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The vulnerable side of science

SCIENCE is about people, the ideas they have, how they go about testing these ideas and how they persuade other people to accept them. Science is therefore largely concerned with communication.

Hardly the sort of definition that would get into a dictionary, which is more likely to speak of science as knowledge obtained by observation and experiment, tested, systematised and brought under general principles. Organised knowledge doesn't at first sound a very personalised affair—it has too obvious associations with the certainties of the periodic table and suchlike, or the neatness of textbooks. Yet every thinking scientist knows that just as 'coal' means a neatly stacked cellar of the stuff to the householder, but something highly personal, both friend and enemy, to the miner at the coalface, so 'science' is not only a neatly stacked cellar but also a complex and messy affair, a very human business with all its successes and failures, argument and consensus. This side of science is relatively poorly understood by the non-scientist. And even scientists themselves sometimes forget it when constrained to write short scientific papers or deliver ten-minute talks in which there is only room for the reporting of success, not the detailing of blind alleys, tortuous discussions, uncomprehending referees.

Because science is so much dependent on free and easy communication amongst its practitioners, anyone who interrupts the channels of communication used by scientists is capable of doing immediate and serious damage not just to those closely involved, but also to the advancement of science. Neutralise a fertile thinker in informal contact with tens, even hundreds of other scientists, and no-one else will be able to fulfil anything like the same role. And neutralising doesn't involve anything as vulgar as demolishing a particle accelerator or breaking up a laboratory. It simply means making one person difficult to get hold of and denying that person the normal means of communicating with the outside world. There are plenty of ways, both subtle and unsubtle, of achieving this, and the demonology is not restricted to governments.

It is, thus, encouraging that 1977 has seen a growing awareness by scientists of the importance of human rights issues, and a gradual realisation that talk of freedom of expression and interchange of ideas in high-flown and little-read international documents need not necessarily be meaningless but could be a lifeline, however fragile, to a beleaguered scientist. The report early in the year by the Council for Science and Society on scholarly freedom and human rights must be reckoned a major milestone in providing scientists with the necessary theoretical support to their practical actions and now, with the Belgrade review of the 1975 Helsinki European security conference drawing to a close, it is reasonable to wonder whether there are any signs that scientists are gaining or losing in their quest for the right to express themselves and communicate with each other.

Obviously there is no measure of such a complicated thing as freedom for scientists to be part of a global community, but even so there are few grounds for believing that things are getting better. Nor is tampering with scientists, and therefore with science, the preserve of any one power bloc or group of nations; there is gloomy news from many parts of the world. And lest it be thought that Western countries can be smug and self-satisfied in these matters, it should also be said that commercial secrecy and national security are often the justification by which *bona fide* scientists are gradually worn down into conformists, afraid to speak out on matters where they have high technical competence.

On the other hand there are favourable signs. Within the next few months it is expected that ICSU's Committee for Safeguards of the Pursuit of Science will come forward with some new proposals, and it is hoped that these will encourage the establishment of some centre to which scientists may turn for assistance or to which malpractices may be reported.

1978 marks the thirtieth anniversary of the adoption by the United Nations of the Universal Declaration of Human Rights. It could well be the year in which the simple, yet very vulnerable needs of the scientist at last get a heavyweight defender. □



Stalin's scientific deputy addresses dissident meeting

THE inclusion of a symposium on scientific research in the Venice Biennale on culture has been described as an "act of considerable courage". Since the general theme of the 1977 Biennale was "cultural dissidence" it was hardly surprising that the round table on scientific freedom should include many who have shown considerable physical and moral courage in the defence of academic liberty. Voronel, Turchin, Plyushch, Dediu, Zhores Medvedev, the Papiashvilis, the Chudovskii brothers—the list of speakers reads almost like a role of honour of the dissident movement in science. Moral courage of no less an order, however, was shown by Dr Arnost Kolman, who, to an audience of active campaigners for scientific liberty, delivered a paper that, under the unassuming title of "The Adventure of Cybernetics in the USSR" described his part in Stalin's campaign to destroy free scientific progress, and replace it by a special party-orientated science.

Dr Kolman, at 85, is a living link with Lenin and the early days of Soviet power. His personal philosophy still remains Marxism, which he sees as having become under Stalin "the obedient servant-girl of political power". His picture of his destruction of science during those years is a horrifying one, since, he explained, it was carried out not by cynical careerists, but by people acting in good faith, but incompetent in the field in question.

Thus he, himself, trained in maths and physics, campaigned during those years against the prevailing philosophy, in defence of relativity, quantum physics, and mathematical logic. In life sciences, however, and particularly in genetics, he was fully prepared to accept the party dogma which pronounced genetics a pseudo-science. He

described how the editorial board of the philosophical magazine, *Under the Banner of Marxism*, of which he was a member, were given a mere three months to familiarise themselves with the theory and problems of heredity—and then to take up the battle against "mendelo-morganism", championing the "innovatory ideas" of Lysenko.

Kolman's paper was not only absorbing—as the *mea culpa* of a notable figure has always been fascinating from the days of Augustine of Hippo onwards—it also set the tone for the whole symposium by stressing the danger inherent on state interference in science. Zhores Medvedev gave a most lucid account of Soviet science policy during the last 60 years, several papers dealt with more recent events in Czechoslovakia (Frantisek Janouch, L. Durovic, and O. Poupa) and in Romania (Mihai Dediu). Kolman's revelations showed, however, that such interference need not necessarily be effected by bureaucrats or party apparatchiki, but by persons who genuinely believe that in doing so they are serving their country and the cause of scientific progress.

Against this background the more theoretical papers took on a significance transcending the current situation in the USSR and Eastern Europe. John Ziman's masterly exposition of the ethical principles underlined the concept of scientific liberty. Giorgio Bert on "Medical Science as an Ideology", Mark Popovskii on "Controlled Science", and Valentin Turchin's enquiry into the correlation between scientific training and involvement in human rights, all raised fundamental problems of the moral responsibility of scientists everywhere.

Likewise, in the special session on the political misuse of psychiatry, Dr Sidney Bloch noted that the revelation

during the last few years of Soviet malpractices had caused many psychiatrists throughout the world to reassess their personal ethical standards.

A number of speakers, in particular Giacomo Morpurgo, stressed the need for rapid and accurate dissemination of information of scientists subject to restriction and deprivation of academic liberty. The work of Amnesty International did not go unnoticed; but it was stressed that Amnesty campaigns only for those actually in prison. Owing to the rapid advance of science, exclusion from professional activity for more than a few months may produce irreversible academic "death". Hence the intervention of the world scientific community at the initial stages of academic restriction is a matter of considerable importance. A prime example of what can be achieved was the worldwide outcry against the campaign to expel Sakharov from the Soviet Academy of Sciences in 1973. Reports of specific efforts were received from Jeremy Stone (Federation of American Scientists), Tania Mathon (Solidarity Committee of French Scientists) and Goran Borg (Swedish Committee for the Freedom of Science).

Repeatedly throughout the symposium it was stressed that, although the theme of this particular meeting was repression and dissent in Europe, the problem of scientific liberty is indivisible and transcends all national frontiers. It is an integral part of the world-wide problem of human rights: if the cases of scientists command particular attention, it is because in this post-atomic age, the outcome of state misuse of science and technology can threaten the future of the entire world. The ethical responsibilities of scientists to their profession and to their colleagues are accordingly proportionally great.

Vera Rich



Arnost Kolman: a living link with the early days of Soviet power

Sakharov warns of subtle pressure

The Soviet physicist Andrei Sakharov sent a personal message to last week's Venice Biennale. The following is an extract from his message:

"Liberty of opinion and exchange of information are of great importance throughout the world. . . . The new climate of international relations, called detente, has led to even more complex and varied links and communications, hence not only have the possibilities of positive influences been increased, but also the danger of the diffusion of more negative characteristics. This has increased mutual responsibility. . . . I consider the initiative of the Biennale to be very important, dedicated as it is to the problem of dissidents and non-official

creativity in the socialist countries. I myself belong to this scientific sector in which the ideological pressure of the state is nowadays not expressed entirely explicitly. . . . The epoch of the ideological struggle against the theory of relativity, quantum mechanics and cybernetics—the most shameful page in Soviet science . . . fortunately belongs to the past. . . . (Now) perhaps the most important thing is the humiliating position of the intelligentsia in the whole country, which is shown particularly in the inadmissible misery of the two most wide-spread intellectual professions—doctors and teachers—and in the total subjection of the intelligentsia to bureaucratic party control".

New defence science chief offers cash to UK universities

UNIVERSITY and polytechnic scientists should become more closely involved in helping to determine strategies for defence research, according to Professor Ron Mason, the newly-appointed chief scientist to the UK Ministry of Defence.

Even five years ago, such a statement would have generated hostility from university colleagues and students alike. Both were concerned—for different reasons—with intrusions into the “purity” of research. Yet when Professor Mason’s appointment was announced at a meeting last week at the University of Sussex, where he has been professor of chemistry since 1971, even a student representative present greeted it, he says, with applause.

With the continued squeeze on university funding, and a growing acceptance of department-sponsored research, Professor Mason claims that there now exists a “more relaxed view” in universities about accepting military funds than a few years ago.

As chief scientific adviser—considered by many to be the highest position that any British scientist can hold in government—one of his main tasks will be to act as an interface with the scientific community. And the new atmosphere should, he feels, make this easier to achieve.

“One of the first things I want to engage in is a discussion on the relative balance of intermural and extramural research, and personally I would like to see a substantial increase, so broadening the base of expertise and advice,” Professor Mason said this week.

He emphatically rejects the notion canvassed by the Campaign for Nuclear Disarmament and others that university scientists should refuse to carry out military research on principle. A scientist’s responsibility should be to produce first-rate research in pursuit of aims determined by the conventional political processes.

“The proviso, of course, is that all research carried out on Ministry of Defence contracts should ultimately be publishable in the normal way; as a university man, I would be worried if an academic institution accepted any restrictions on publication.” Professor Mason has himself in the past received funds from the Microbiological Research Establishment at Porton Down in support of certain aspects of his work on ion transportation across cell



Ron Mason: “a more relaxed view” on military research

membranes, and feels no qualms at having done so.

“Another reason for wanting closer contact with scientists in universities and polytechnics is to open up discussions about long-term trends in defence research. And since these involve a mix of the scientific and technological with economic and political factors, a broad interdisciplinary discussion of objectives is required for which the ministry research establishments are not equipped.”

Professor Mason is no stranger to the world of science and technology policy, having been a member of the Science Research Council from 1971 to 1975, and chairman of its science committee for three years. He has also been a member of the requirements board of the Department of Industry since 1973.

One area in need of investigation in his new job is, he says, the systematic under-recruitment of scientists and engineers by the Ministry of Defence that has taken place in recent years.

“Whether a reluctance to take up a career in military research is due to the unattractiveness of Civil Service salaries in general, or of this type of work in particular, is at present unclear. But it might be a good idea for me to go out to talk to people in universities to help find the reasons for this situation.”

Professor Mason admits to being slightly “bewildered and overwhelmed” by the scope of his new job, which he takes up on 1 March, 1978 on a three-year secondment from the university.

He is convinced that the key to its success, at least in developing links between the scientific community and the defence establishment, lies in opening up discussion about military research; but whether closer liaison is acceptable to either party remains to be seen.

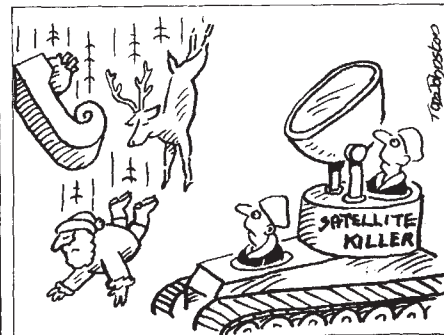
David Dickson

Soviet beams over Sweden?

Is the Soviet Union developing a charged-particle anti-satellite weapon? Reports earlier this year listed evidence that tests of such a weapon had been carried out at a research facility near the Soviet city of Semipalatinsk, and described the debate raging between the US Air Force, convinced that the tests were being carried out, and the more skeptical CIA. A Swedish scientist has now published some findings that will add to the speculation.

Dr Lars Erik de Geer of the National Defence Research Institute detected traces of radionuclides neptunium-239 and molybdenum-99 in the atmosphere over southern Sweden on five occasions during the first half of 1976. The same atmospheric samples also contained small amounts of fission products iodine-131 and barium-140. On the first two occasions—in late February, and March—the prevailing winds had been from the east during each period of three days when the nuclides had arrived. On the other three occasions—in April, May and June—the winds had blown from the east on at least two days in each week during which the nuclides came. Dr de Geer thus postulates that “the material arrived in Sweden by way of southern Finland or western USSR and the Baltic Sea”.

What could have caused such activity? The short answer is: no known source. The amounts of neptunium and molybdenum detected are compatible with the ratios found in debris that circulates for a few weeks after the explosion of a nuclear bomb. But, as Dr de Geer points out, if these nuclides resulted from some fission process, it is strange that other short-lived fission products (such as tellurium-132) were not also detected. Neither is the composition of the samples consistent with discharge from an ordinary nuclear power reactor. He wonders whether some laboratory experiment involving fresh fission products could have been responsible; but a check on research laboratories in Sweden, Denmark and Finland showed that no such work was in progress during the period in question. The



most exotic explanation—that the nuclides were produced by charged-particle beam experiments in the Soviet Union—seems to fit in that the observations were made at the same time as the experiments at Semipalatinsk were reportedly started. According to meteorological trajectories, however, nuclides from this area of the USSR could have been carried to southern Sweden in March, April and May, but probably not in February or July. This means that, although the nuclides detected in February were deposited by an easterly wind, they probably came from a source other than Semipalatinsk. Given the nuclides' short half-lives (ranging from 2.35 days for neptunium-239 to 12.79 days for barium-140), and the distance from Semipalatinsk to Sweden (about 4,000 km), the winds carrying the nuclides would have to have been strong. Although meteorological data suggests that the winds probably would have been strong enough, sufficient doubt remains for Dr de Geer to cite this as a factor against the Soviet source.

Dr Kay Edvarson, of the National Institute for Radiation Protection, comments that it is impossible to pinpoint a unique source for such activity. "We have fairly extensive surveys made now on releases from power reactors, and Dr de Geer's findings don't really fit any reactor types known to us. The National Defence Research Institute quite often detects some radioactivity which can not have come from a nuclear test. These samples are generally assumed to have come from small, localised sources such as a hospital. But it is impossible that the sort of activity Dr de Geer detected can have come from such a source. On three occasions the nuclides were detected at several sampling stations in southern Sweden, so it's highly improbable that the source was localised. It has to be reasonably far away, and strong. If it had been a strong source somewhere in the Nordic countries or West Germany, we would have heard about it." He points out that the probability of detecting the sorts of samples now under discussion is small.

"They would be masked by fairly fresh debris from a nuclear test", he says, "and the release time has to coincide with the wind pattern".

Dr Bhupendra Jasani, of the Stockholm International Peace Research Institute (SIPRI), says it is difficult to think of any sources other than those mentioned by Dr de Geer which might have caused the activity. "I am very skeptical of those who claim that the Russians have a charged-particle beam weapon in operation", he says. "However, the Soviets are pioneers in accelerator physics and accelerators, and they could well be working on such weapons—just as the Americans may be." Dr de Geer's report, published in *Science*, quickly drew a statement from the Pentagon questioning the hypothesis of the Soviet source. But, as Dr de Geer points out, "I am not saying that the nuclides came from charged-particle beam weapon tests in the USSR. It is simply that, as no other source seems to fit, the possibility that they did has to be considered."

Wendy Barnaby

Sweden follows US example on aerosols

SWEDEN is to become the first country in Europe to ban the manufacture and use of chlorofluorocarbon (CFC) propellant gases in aerosol sprays.

The ban, which will take effect in 1979, reflects public concern at the possibility that such gases may deplete the stratospheric ozone layer, hence potentially reducing the atmosphere's ability to screen ultra-violet rays, and leading to an increase in skin cancer.

In the US, fears that the extensive use of CFC gases could cause a decrease of ozone of up to 14% over a period of 100 years have already led to a similar ban, the result of a joint decision by the Food and Drug Association, the Environmental Protection Agency, and the Consumer Product Safety Committee.

Sweden is the first country in Europe to follow the American example. The Swedish Government's action is based on a recommendation of its Products Control Board that a licensing system should be introduced for aerosols, and that such licences be withheld from most aerosols using CFC gases.

The manufacture and import of such sprays—excluding certain products required for special uses such as medicine—will be banned from the beginning of January 1979, and their sale from the following June.

The move has already brought reaction from a number of Sweden's trading partners in the European Free Trade Association (EFTA), concerned at the economic impact it may have. Finland,

for example, which exports many aerosols to Sweden, has asked Stockholm either to withdraw the ban, or to extend the timescale considerably.

The Finnish request is based on the position held by the majority of Euro-



"It's an aerosol for getting rid of Finnish aerosol salesmen"

pean countries that although CFC aerosols represent a theoretical danger to the ozone layer, no substantial evidence has yet been produced to indicate that any significant depletion of ozone does indeed take place.

Widespread public controversy has surrounded the issue since the possibility of such depletion was raised by

Rowland and Molina in 1974. It is this concern that has led to the current ban in the US, where many manufacturers are turning to the use of hydrocarbon gases as an alternative.

No theoretical model of the chemical interaction between ozone and CFCs in the atmosphere, however, has yet produced an adequate representation of what actually happens. In particular, most models rely on a one-dimensional interaction, and are hence unable to take into account the full three-dimensional movement in the stratosphere.

In view of the scientific uncertainties, countries such as Britain, Germany and France have decided that more research is needed before a decision about whether or not to ban CFC aerosols. The EEC has recommended that a review of the situation be made in the second half of 1978, and has also commissioned the UK-based consultants METRA to carry out an economic impact study on the effects of a ban.

The Swedish government has therefore decided to break ranks with other European countries, and in particular its Scandinavian neighbours. However the decision is likely to be welcomed in the US, where FDA commissioner Donald Kennedy said earlier this year that since the threat of ozone depletion was a global problem, he hoped other countries would follow suit.

An official of the Swedish Department of Industry said in Stockholm last week. "The Swedish philosophy is that this will take a long time, and someone has to take the lead."

David Dickson

Bangladesh reviews science policy

MODERN science and technology was introduced to the Indian sub-continent by the British during the late 19th century. It developed in only a few areas of present day India, no significant development taking place in those areas which now make up Bangladesh and Pakistan. A national policy for science and technology development there was initiated as late as the 1960s. Planning and executive agencies were subsequently formed and some institutions for scientific and technological education and for research and development were established. Over the last ten to fifteen years an indigenous scientific and technological infra-structure has begun to be built up in Bangladesh.

The need for an integrated science and technology policy in Bangladesh has led to the formation of the National Council for Science and Technology (NCST) to advise the government on formulating policy, and planning, coordinating and evaluating science and technology development. In concrete terms the NCST determines national objectives, formulates strategy and identifies areas where science and technology can contribute to national development. The talks also include coordinating and evaluating scientific and technological programmes in various sectors, and the development and motivation of scientific and technical manpower.

The government of Bangladesh has now undertaken economic development programmes to raise the standard of living of the people and improve socio-economic conditions. Implementation of these programmes needs specific technological capabilities. Any assessment of future possibilities and formulation of appropriate development strategies must be based on an analysis of national strength and weaknesses which should take into account the potential contribution that science and technology can offer. Science and technology policy for both short and long term strategies, thus, constitutes an integral part of the country's overall development plan.

With a view to planning and programming scientific and technological developments effectively, the NCST has undertaken to establish a data base on manpower, and the funding, management and facilities for the existing scientific and technological infrastructure. This would involve surveying existing scientific and technological personnel and stock taking of the research institutes, laboratories, workshops and supporting facilities. The current programmes in these institu-

tions are to be reviewed and plans for the development of existing scientific and technological institutions are to be made on the basis of the results of these surveys and reviews. Further, NCST intends to undertake studies and make recommendations on the organisation and modes of financing of the research councils, institutes and other supporting facilities.

On the development of human resources in science and technology in Bangladesh, NCST proposes to review the existing education and training programme and suggests appropriate measures for their expansion and improvement. Recruitment and promotion rules for scientific and technical personnel with appropriate measures for proper incentive and motivation. The government has already instituted a fellowship programme for science, engineering, medical and agricultural graduates who cannot find suitable employment inside the country or experts working abroad but interested

in joining the institutions of Bangladesh.

NCST intends to undertake a comprehensive scheme for preparation and production of science and technology text books in Bengali and reference books in both Bengali and English up to the highest level of education and to advise the government on its phased implementation. Popularising science and technology and disseminating scientific knowledge among the public, particularly on the human implications and social impacts of science and technology will be one aim. In the long term these studies should lead the government and other concerned agencies to formulate the philosophy, guidelines and workplan for a course of action.

Bangladeshi science and technology policy also envisages longterm studies on the development of scientific and technological potential, appropriate or alternate technologies, policy guidelines for international and regional cooperation and promotion of associations, academic and professional bodies and human rights for scientists.

M. Kabir

FAO conference embraces third world

WHEN, in January 1976, Edouard Saouma, a Lebanese Christian, took over as Director General of the Food and Agriculture Organisation (FAO), there was a good deal of headshaking among the old hands in international agriculture, and when he announced his policy for improving the efficacy of the organisation, there was even more heart-searching among the senior staff. His policy was aimed specifically at more direct contact with ministries of agriculture and development in the Third World countries.

The FAO biennial Conference, which ended early in December at the headquarters in Rome, must have given Saouma a good deal of satisfaction. After two years of his regime, government after government expressed approval and encouragement for the policy at which many of their representatives had looked askance early in 1976. Nor did this approval come only from the Third World countries towards whom the new policy has been especially directed.

The most far-reaching of Saouma's ideas has been the Technical Cooperation Programme. Seen initially by many as a duplication of effort with the UN Development Programme (UNDP) this has now proved its ability to provide small-scale assistance for specific projects at very short notice—something the cumbersome machinery of UNDP has never been able to provide. In its first year, 190 projects have been approved under this programme. The success of

this scheme certainly owes a good deal to another innovation, much criticised when announced: the appointment of FAO representatives to certain countries, again in order to provide direct and rapid contact in critical areas.

Two other activities came in for special approval from many delegates in Rome. One was the Seed Improvement and Development Programme, fundamental to increased production in the developing countries. The other is the campaign against post-harvest food losses—something for which every FAO Conference has asked since the organisation came into being. Since it is estimated that cutting such losses by half could save up to \$7,500 million a year in the foreign exchange for the developing countries, the amount allotted for initiating this campaign, a mere \$10,000, seems modest indeed. It is hoped to double this with external contributions.

Referring to the 1979 World Conference on Agrarian Reform and Rural Development, Saouma said that he wishes to "avoid confrontation, rhetoric and confusion"; many observers must have noted that he certainly managed this at his own Conference, at a time when politicising and backbiting have come to be a normal part of such major international meetings. This is perhaps another good augury for his ability to control the spending of the \$237,377,000 he is asking for his regular budget for the biennium 1978-79.

Peter Collins

Australian election leaves uranium issue confused

LAST weeks' general election in Australia scarcely changed the massive majority held by the Liberal and National Country Party coalition government. Nor has it changed the odds on whether or when Australia is to become a major exporter of uranium.

At first sight this observation may seem paradoxical—even perverse. Surely such a total victory must have fortified Mr Fraser and his government in their confrontation with the opponents of uranium mining? This certainly seemed to be the view of the uranium mining companies expressed through their lobby organisation, the Australian Uranium Producer's Forum. Within hours of the election result being known the forum's chairman declared that the Fraser government now had a mandate for its uranium export policy. Unfortunately even if one accepts the dubious mandate the mining companies' view is without foundation.

While the government parties polled about 48% of the national vote, the combined vote of the two main parties advocating a moratorium on uranium mining, the Australian Labor Party and the Australian Democrats, was almost 50%. Furthermore, it would be hard to argue that the uranium issue was at the forefront of most voters' minds as they went to the polls. Although it had been seen, before the event, as a crucial issue in a December general election, in the event uranium received relatively little attention during the campaign. The government parties, in particular, seemed to go almost out of their way to avoid discussing uranium and nuclear power. In an opinion poll conducted eight days before the election, one of the more respected polling organisations found that only 5% of voters considered uranium export to be the most important election issue. Thus the election result is in no sense a positive endorsement by the majority of Australians of the government's policy on uranium.

Government undeterred

This fact is unlikely to deter the government from attempting to claim a mandate for its policy. And the election result is certain to increase its confidence in seeking a confrontation with those who oppose it, which means, principally, individual unions or the trade union movement as a whole. There has been considerable speculation about when and how such a confrontation may occur. In the transport

of yellowcake, unions have already taken industrial action on uranium.

The most crucial of the unions are almost certainly those in the building and engineering industries, whose members will have to be involved in constructing the mine and mill. These unions are mostly large, well organised, and strongly opposed to the mining and export of uranium. They seem certain to be in the vanguard of any confrontation with the government. While it is obvious that a great many factors will determine the outcome of a confrontation, should it occur, the support which the unions receive from the labour movement as a whole and from the general community will clearly be of great political importance.

There is now a large and articulate opposition to uranium export, co-ordinated nationally through the movement against uranium mining. While not yet as large as the movement opposing the Vietnam war was at its peak, the opposition to uranium mining appears to be both better organised and politically more sophisticated.

Up till now Mr Fraser has treated the uranium issue with great circumspection. His failure to use it as an election issue could be taken to indicate uncertainty about the popularity of his policy. In explaining the policy, he emphasised that following the reports of the Ranger Uranium Environmental Inquiry (otherwise known as the Fox reports) he has demonstrated in both word and action the importance he attached to improving safeguards against nuclear weapons proliferation. All these moves may have been effective in reassuring uncommitted opinion on the uranium issue; though if Mr Fraser was hoping that he would also placate his opponents, he must have been disappointed.

However, there are now signs that the government is being less cautious in pursuing its uranium export objectives. For example, it has so far failed to provide the necessary finance and administrative support for the team appointed to carry out environmental research and supervision in the Alligator Rivers region of the Northern Territory, where the major uranium deposits are located. While the hold-up may simply be the result of bureaucratic inertia or parliamentary delay, the opponents of uranium mining are inclined to see it as presaging the breaking of promises on environmental protection.

Much more important, at least in the international context, was the state-

ment in the last week of the election campaign by the Deputy Prime Minister and Minister for National Resources, responsible for uranium in Mr Fraser's pre-election government, Mr Anthony. He announced that the government was reconsidering its policy of prohibiting the reprocessing of spent reactor fuel made from Australian uranium, and does not wish to rule out reprocessing by 'acceptable' countries. This change in policy would make it difficult for the government to continue to argue that by exporting uranium it was furthering President Carter's policy of preventing reprocessing and the plutonium economy by providing ready access to supplies of nuclear fuel.

Mr Anthony has consistently been far more blunt than Mr Fraser in his support for uranium mining and his statement may not have the support of Mr Fraser. In the immediate aftermath of the election it is not certain that Mr Anthony will retain responsibility for uranium development in Mr Fraser's new government. However, his statement undoubtedly reflects the problem the government is having with its policy on proliferation. President Carter's policy on nuclear fuel reprocessing is clearly in some difficulty, and may be abandoned. More important from the Australian government's point of view will be the firm commitment to reprocessing by its major potential uranium customers: Japan, West Germany, and the UK.

Economic question

Finally, there is the question of the economics of uranium mining. Canadian officials involved in making decisions on the development of the new uranium prospects in Saskatchewan recently expressed amazement at what they considered to be highly optimistic expectations of both sales volume and price of uranium held by the Australian government and mining companies. The uranium debate in Australia does not so far appear to have comprehended the considerable down-turn in uranium market prospects over the next ten years or so, which has taken place recently. When the Australian people realise that an immediate start to uranium mining may not bring the great economic benefits they have been led to expect, they may be less willing to support the government in any confrontation that may occur. It is noteworthy that one of the few seats to record a significant swing against the government was Northern Territory, where much publicity has been given to the possible economic benefits of uranium mining. There is still a very long way to go before Australia becomes a major uranium producer.

Hugh Sadler

correspondence

Letter from Argentina

SIR,—I welcome the concern Louise Harel, Jose Uriel and Jean-Claude Salomon show to my countrymen (3 November, page 8) by inviting professionals not to participate in the twelfth International Congress of Cancerology to be held in Buenos Aires, in October 1978.

However, while I believe that sincere international solidarity can be of great help in the fight for universal human rights, and while I welcome the writers' desire to see democracy upheld, I believe their proposal in this case is misguided.

Many countries are suffering from terrorism. In Argentina scientists, professional people, students, workers, policemen and the military, priests, women and children have been affected. It is true that there is a long list of people who have disappeared or been kidnapped and that some have been forced to emigrate by intimidating threats and torture. But action is also being taken against all kinds of terrorism. President Videla has repeatedly declared that the state is the only force charged with the security of people and that any illegal repression will be severely punished. He has also expressed the need for exchange of opinions if Argentina is to be based on a renewed, stable and progressive democracy.

These concepts are shared by the Permanent Assembly for Human Rights and by the Argentine League for the Rights of Man, both functioning legally. Political personalities, cultural and scientific representatives, as well as others from all branches of activities, publicly condemn the terrorist violence and call for an end to persecution and arbitrary repression.

But is a decision to boycott the International Congress an appropriate way of expressing solidarity? Is it in effective way of helping us reach a democratic goal? I think not. One of the objectives of terrorism is to disorganise the life of a country, to create a climate of intimidation and to isolate the nation from the international community. A boycott of the Conference would contribute to this isolation and would favour the plans of terrorists to promote chaos. The fight against terrorism should stem from the organised way of life of the country itself, which will break up antisocial manifestations. The government has assumed this

responsibility by proclaiming its monopoly of repressive actions against terrorism. It is also the responsibility of the democratic forces which speak out for the right to work in peace and liberty. This is what scientists, professional people, workers and their institutions and associations are doing. All these activities are proceeding in spite of economic restrictions and social violence and they should be supported.

No matter how great the concern of our colleagues abroad, Argentinians are far more concerned about showing support for security and peace. It is precisely to show solidarity with our efforts that foreign scientists should come to our country, should acquaint themselves with our complex reality, should make their contributions towards the fight against cancer and should acquaint themselves with the Argentinian work on the subject.

I am making this appeal as a scientist who has been dismissed from his position in the National Council for Scientific and Technical Research (CONICET), for "service reasons", without any justification. I have not emigrated, neither have most of those affected. I have asked for reconsideration and I will continue my fight for justice. I am going to participate in the International Congress of Cancerology with my modest contribution under such difficult circumstances. I am confident that the patriotic forces of our country, both military and civil, will find a way of effectively restoring democracy. All honest Argentinians are striving for this end. The presence of foreign scientists at the Congress will be an expression of respect and solidarity with our human and scientific endeavour when we need it most.

EMANUEL LEVIN

Buenos Aires, Argentina

Go to Argentina

SIR,—Once more the European scientific community is threatening to boycott the scientists of a specific country because of the type of government they have ("Petition for Argentinian scientists", 3 November, page 8). I think this is unfair to the great majority of scientists and students of the particular country. I would like to comment on this problem by taking the example of Chile, my native country.

As a small country with a low population, mainly supported economically by copper exportation, Chile used

to have the reputation of being an example of democracy in a continent where, together with two or three other countries, it was the exception. Its universities had excellent research groups in many disciplines able to compete with scientists from much richer countries.

All this was jeopardised by the 1973 military "putsch"; the research effort was severely affected but not killed (see "Science in Chile since Allende", *Nature* **265**, 486, 1977). Many Chilean scientists left the country, some because of direct persecution, others because of political ideas or moral convictions. The first of these groups was composed of scientists who had key positions during the 1970-73 period, or who were considered to be important by the "new lords". Many of these people were imprisoned and then went abroad through embassies. In the second group are many scientists who had already been abroad and for whom the possibilities of obtaining good positions in the USA and Europe were favourable.

Some university departments had to close down because all the teaching staff had left. This was the case for disciplines like physics and mathematics. In the field of biochemistry, however, an important number stayed, although many left. Who are these women and men who did not leave?

Are they all fascists as is generally assumed, mainly in Europe? One thing is clear to me: the longer they remain isolated from the rest of the world, the more likely it is that they will become convinced by the government of the so-called "general Marxist con-fabulation against Chile". There may be a small fraction of native academics that is sympathetic to and even enthusiastic about the "junta", but most of the Chilean scientific community is made up of convinced democrats who have supported leftist or left of centre political positions. For professional or familial reasons they have stayed in Chile.

What happens when an invitation or the opportunity to visit Chile is open to a foreign scientist? Immediately thousands of voices full of fury are raised to protest against such support of the authoritarian regime. This is extremely evident among European unions or intellectuals. Of course, other annual exchange programmes organised by certain European governments with other dictatorship-ruled countries do not raise such a reaction.

The attempt to isolate Chile reminds me of a similar attempt to isolate Brazil a few years ago. Although the Brazilian government is still very authoritarian, scandals are no longer caused when foreign scientists are invited to Brazil. Moreover, the great majority of the Brazilian scientists are opposed to the military government (although many of them returned to Brazil after the military took over, because of very important salary rises).

The main victims of this isolation, at least in the case of Chile, were the common people and more specifically scientists and students. The Chilean government, less stupid than generally thought, has used the argument of "all against us" as a powerful nationalistic tool. It seems aberrant to me that while scientists from all over the world are being persuaded from going to Chile, a superpower, which originated and supported the isolation campaign, is engaged in an exchange of political prisoners with the Chilean government, giving a tremendous popular credit to the military junta inside the country. A visit of all Nobel laureates to Chile, would not strengthen the military as the exchange of prisoners did.

Attending an international scientific meeting or visiting a Chilean or Argentinian university is not synonymous with supporting the military government of these countries. On the contrary it is a unique opportunity to contact scientists and students and to encourage and help them to overcome the scientific and moral isolation they suffer.

We have to remember the case of Spain, a country which until a few years ago had the same type of government as Chile and Brazil now have. As long as it was isolated from the world no hope of ending the dictatorship existed. As more and more foreigners entered the country, an irreversible flow towards democracy started.

No tourist boom can be expected in Chile or Argentina, but scientists and academics passing through can be an indispensable link with students and scientists. Without this bond, they may be convinced by the chauvinistic arguments used by the government each time a world campaign is organised against it.

This is a plea not to support the South American dictatorships or to ease the criticisms against them but to maintain a link between their scientific communities and the rest of the world. This may be the unique possibility of keeping alive the result of years of effort, and the only hope of evolution towards the revival of democracy in these countries.

SIMON LITVAK

University of Bordeaux France



Competition 15 asked for an appropriate quote for our front cover. A good varied entry with an honourable mention to D. Irwin (Boston) for "*Pereant qui ante nos nostra dixerunt*" (May those perish who have said our things before us). But £10 goes to the Mammalian Development Unit, University College, London

Technik or technics

SIR,—On most topics, I would not set out to disagree with those who write to support me. However the question is too important to let go: it concerns the separateness of the German-language notion of a third culture of *Technik*.

Rey and I (1 September, page 2) argued that the idea of *Technik* represents a missing concept in English, whereas Cavalier-Smith (20 October, page 646) would like us to follow Lewis Mumford instead and revive the English word "technics". We avoided "technics" because Mumford got things very wrong in the book referred to, *Technics and Civilisation*.

Notably he argued for a distinction between the "neotechnics" of the age since about 1880 or so, and the "paleotechnics" which went before. Of the period just before neotechnics, he wrote that its inventions "came into existence, for the most part, without its [science's] direct aid"; whereas later in the "neotechnic phase, the main initiative comes, not from the ingenious inventor, but from the scientist who establishes the general law: the invention is a derivative product".

Detailed investigation does not support this viewpoint, or the distinction between ages of manufacture on which Mumford's thesis is founded; science has always influenced manufacture, but never controlled it. Neither does detailed investigation support the viewpoint of R. J. Forbes, the author of *Man the Maker* (1950, Shuman), another hero of the historians of "technology". He writes his subject up, most misleadingly, as an account of the exploits of "applied science" through the "conquest of nature". A reliable book of the influence of *Technik* on the culture has yet to be written.

I am absolutely at one with Cavalier-Smith, however, that there is only harm to be done to science in the long term, through a blurring of the distinction between science and the technical

who communally turned up: "Is this," I cried, "the end of prayer and preaching? Then down with pulpit, down with priest, and give us Nature's teaching!" (Whittier)

Competition 16. To the uninitiated, many scientific pictures are ambiguous: a section through a cell could equally be a slice of a lunar rock. Readers are invited to give a misleading caption to any picture that has appeared in, or on the cover of, a recent *Nature*. £10 to the winner. Entries to Competition 16, *Nature*, 4 Little Essex Street, London WC2 by 1 February 1978.

functions of manufacture. One vehicle for this harm has been the ghastly construction called "technology"; the only "-ology" in history to make useful, bulky artifacts as a primary output, a word which outsiders to manufacturing use when they want to hide their ignorance of it.

M. FORES

London, UK

Sunflower's siesta

SIR,—Observations on the sunflower's night life (8 September, page 102) prompt me to put on record a piece of its daylight behaviour which I have never seen described before. In Turkey, in July this year, I consistently saw that at 1 pm on bright days the flowers all faced the sun but by 2.30 pm all heads had turned away and faced north. At about 5 pm they again began to face the evening sun and ended the day facing west.

Perhaps even the sunflower finds the Turkish afternoon summer sun a bit hot and has a thermal switch overriding the sun-tracking mechanism, so that it can have a siesta. Is the siesta phenomenon unusual, or do sunflowers always behave like this in hotter climates?

ROBERT G. MILNE

Torino, Italy



news and views

Immune surveillance revisited

from R. W. Baldwin

THE immune surveillance theory as developed by Burnet (*Immunological Surveillance*, Pergamon Press, Oxford, 1970) proposes that a central role of the immune response is to provide a natural defence against cancer and this was later developed to emphasise the importance of thymus-dependent lymphocytes. It was, therefore, more than a little disconcerting to find that congenitally athymic (nude) mice do not have an increased incidence of spontaneous cancers and they are no more susceptible than conventional mice to chemical carcinogens (reviewed by Stutman, *Adv. Cancer Res.* **22**, 261; 1975).

It is axiomatic, however, that nothing in tumour immunology is simple and it has recently been proposed that whereas T lymphocytes may be an important component of the responses induced by immunological manipulations, such as immunotherapy, they may not have a central role in more natural conditions such as those involved in recognising clones of transformed cells. Instead, attention is being focused on so-called 'natural killer cells' since these fit almost exactly the immunologists' design for a surveillance cell in being able to recognise and kill malignant cells without previous sensitisation. The basic observation which drew attention to natural cell-mediated immunity arose out of quite extensive studies comparing the *in vitro* cytotoxicity of peripheral blood lymphocytes from cancer patients and normal donors for cultured cancer cells. Initially these investigations were interpreted to show clear-cut specific cytotoxicity by 'sensitised' lymphocytes from patients, but in later studies by Takasugi *et al.* (*Cancer Res.* **33**, 2898; 1973) and Oldham *et al.* (*J. natn. Cancer Inst.* **55**, 1305; 1975) it became apparent that lymphocytes from normal individuals, who would not be expected to have been exposed to the relevant cancer-associated antigens, were also cytotoxic. Moreover in many

instances the natural cytotoxicity of lymphocytes from normal individuals was even greater than that seen with similar preparations from cancer patients. Natural cell-mediated immunity has since been examined using more closely controlled animal systems and, as in humans, lymphocytes from normal mice and rats have proved to be cytotoxic *in vitro* for tumour cells, especially those of lymphoid origin.

In the mouse and rat, normal killer (NK) cells have been detected in most lymphoid organs, with particularly high activity in the spleen, lymph node and peripheral blood whereas in humans, studies have mostly been restricted to peripheral blood lymphocytes. In mice NK cells develop in the absence of the thymus and appear and disappear in a highly typical manner, reaching peak levels at between 5 and 8 weeks of age. NK cell activity is also under genetic control, allowing the classification of low and high NK cell strains. The position in other species, especially humans, is still unclear, but it has been proposed that NK cell activity may be related to HLA phenotype. The characterisation of NK cells is more controversial and it is this aspect of the problem which leads to most confusion. In the mouse, the NK cell is thought not to be a mature T cell because of its presence in athymic mice, although Herberman and Holden (*Adv. Cancer Res.* **27**, in the press) have suggested that it might be a primitive T cell. The characteristics of NK cells in humans are even less well understood and at present the only point of general agreement is that these cells are not macrophages.

Although it has been implied that natural killer cells may provide the host with a natural barrier against malignant cells, evidence on this point is sparse. The resistance to growth of transplanted tumours, including those of human origin, in athymic nude mice has been correlated with their high levels of natural cell mediated cytotoxicity. This evidence is not sufficiently compelling, however, since

these animals have been reported by Pimm and Baldwin (*Nature* **254**, 77; 1975) to be able to reject tumours through macrophage-mediated reactions. More convincing are studies by Kiessling and his associates (*Int. J. Cancer* **15**, 933; 1975) correlating the growth potential of transplanted tumour cells in different strains of mice with their levels of NK cell activity. Also within one strain Sendo *et al.* (*J. natn. Cancer Inst.* **55**, 603; 1975) found that resistance to a transplanted tumour changed with age in a pattern similar to the known age-dependent variation of NK cell activity. These approaches have been further extended in a study now published by Haller *et al.* (this issue of *Nature*, page 609) which provides more direct evidence for a role of NK cells in suppressing tumour growth. Mice were thymectomised, irradiated and reconstituted with anti-T-cell treated bone marrow cells or foetal liver cells. When lymphoid cell donors of a compatible substrain of high NK cell activity were used, the transplanted tumours were rejected, whereas no resistance was observed in mice receiving cells from donors with low NK cell activity. These findings point to the potential of NK cells in tumour resistance, although whether they have a 'decisive role' remains to be established. In this context, it is pertinent that cells with similar characteristics to NK cells can be isolated from tumour biopsies, and that bacterial vaccines such as bacillus Calmette Guérin (BCG) and *C. parvum* which are being widely used in cancer immunotherapy trials markedly influence NK cell activity. One should guard against a too unitarian view, however, since many of the approaches for enhancing nonspecific immunity against cancer by the use of bacterial vaccines are known to require macrophages. Nevertheless the concept of a nonspecific lymphocyte which recognises aberrant cells is attractive for the immunosurveillance concept and augmentation of these cells could have therapeutic potential. □

Are comets dirty snowballs or dust swarms?

from David W. Hughes

HEATED debates in science are always fascinating and none more so than the long standing one between cometary scientists as to whether comets are made up of a swarm of small, meteoric dust particles or a single kilometric sized icy nucleus. More fuel has been heaped on the fire recently by Lyttleton (*Q. Jl R. Astron. Soc.* **18**, 213; 1977) and Fellgett (*The Observatory* **97**, 23; 1977).

Both authors base their argument on that well worn principle of scientific methodology first propounded by William of Occam in the fourteenth century. Occam's Razor intimates that theories should be based on as few hypotheses as possible. Hypotheses must not be invented merely to explain away difficulties, especially if those hypotheses are not suggested by other features of the phenomena under investigation and cannot be tested in any way.

Swarm or macronucleus?

How does this apply to comets and especially to the debate between the swarm enthusiasts, of which Lyttleton and Fellgett are the most enthusiastic, and the large army of macronucleus supporters? First, I shall explain the two concepts in more detail. The classical view, held by most people up to the early 1950s, is that a comet is a vast swarm of tiny particles separated by very large distances—around 10^{25} particles of mean size about 10^{-2} cm separated by tens of metres. The average comet diameter is about 100,000 km, average mass around 10^{18} g, spatial density less than 10^{-12} g cm $^{-3}$. The icy conglomerate model was formalised by F. Whipple in the early 1950s (*Nature* **263**, 15; 1976) and since then it has gained pre-eminence. Here the fount of cometary phenomena is a single kilometric sized body containing about 25% by mass dust particles, the rest being ices of methane, ammonia and carbon dioxide embedded in H $_2$ O snow as hydrates or clathrates. A comet of mass 10^{18} g would have a nucleus about 6 km across with a density around 1.1 g cm $^{-3}$.

Both these models are hypothetical and as such Occam's Razor seems powerless to distinguish between them. The swarm is so tenuous that it would

have to extend over 10^7 km in the line of sight before it ceased to be transparent. A solid nucleus of length 1 km transverse to the line of sight at 1 AU would subtend an angle of less than 0.002 arcs at an Earthbound observer and is thus way below the resolution of all present day telescopes. So both are unobservable.

Cometary origin

Hypotheses on cometary origin also seem powerless to distinguish between the two models. Swarm comets are supposedly formed by accretion from an interstellar cloud. Particles streaming past the Sun become gravitationally focused downwind, lose energy by inelastic collisions, are retarded to velocities below the escape velocity and then become gravitationally attracted to the Sun, slight perturbations preventing them actually falling into it. Dirty snowball comets are supposedly the remnants of an enormous family of planetismals, some of which coalesced to form the planets Uranus and Neptune. These cometary planetismals were perturbed (by planetary encounters) out of their original orbits to the Oort sphere, a great (but again unobservable!) comet reservoir some 40,000 AU from the Sun, from which they are periodically perturbed by passing stars into orbits with perihelion passages among the planets. Both theories are possible but obviously it requires only a small addition to the interstellar accretion theory to produce icy nuclei anyway. The fact that diffuse interstellar dust and gas clouds are considered to be the parents of stars and planets (and we should at least be convinced that planets exist) does not put this beyond the bounds of possibility. If the planetismal theory is true, all comets are as old, if not slightly older than the planets (giving them an age just over 4.6×10^9 years) and the number of comets in the Solar System must have been decreasing ever since. Fortunately icy nuclei comets can be kept in 'cold storage' in the Oort sphere. Accretion mechanisms have the advantage that the comet family can be enhanced every time the Sun passes through a dust cloud. Here comets can have a wide range of ages.

It is to be hoped that the observations of cometary phenomena can distinguish between the two models. Evidence is needed that points unequivocally at one or the other (or neither).

Unfortunately both models can explain many phenomena with reasonable success. The coma can be formed by desorption from the solid meteoroids in the swarm or by ice sublimation from the nucleus. Desorption is enhanced by the interparticle collisions which take place near perihelion as the individual particles in the swarm move from above to below the median plane of the swarm orbit. Collisions also bring about the emission of light and the release of gases. Sublimation increases as the temperature of the nucleus increases. Meteoroid streams can be formed by the ejection of particles from the nucleus by gas pressure or by the fragmentation of swarm particles.

Phenomena

The problematic phenomena are as follows. The coma generally contracts as the comet approaches the Sun, roughly in proportion to its heliocentric distance, and then expands again as it recedes. A swarm would do this. However, the observation is much more difficult to explain with an icy nucleus. The sublimation of the different icy species would have to have an unusual temperature dependence to fit the observations.

Some comets engender intrinsic forces which change their periods, this change being non-random and continuing from one orbit to the next for tens of years. The periods can both increase and decrease. Lyttleton explains how perihelion collisions in a swarm can result in a loss of orbital energy and an acceleration of the mean motion. But how can the energy of the swarm increase and the period decrease? With a rotating icy nucleus (and what is not rotating in the Solar System?) any delay in the sublimation process would produce a jet force with a component normal to the solar direction and thus accelerate or decelerate the orbital motion, depending on the sense of rotation.

Comets are seen to split up into separate components which move apart with relative velocities of a few m s $^{-1}$. Asymmetrical jet action could spin-up the icy nucleus until it breaks up under the action of centrifugal force. It is difficult to envisage how swarms can split up.

Some comets undergo outbursts, sudden increases in brightness which can be as large as a hundred-fold. In

the case of comet Schwassmann-Wachmann I the occurrence of these bears no temporal association with perihelion passage, as would be expected if the comet was a single swarm of particles. An *ad hoc* increase in the number of swarms in an individual comet helps, but goes against the whole tenet of Occam's argument. Meteor studies indicate that Encke has made thousands of orbits around the Sun and this gives Encke a recent inner Solar System lifetime of at least 10,000 years. Long period comets are expected to have lifetimes easily up to ten times this value. The high surface area to mass ratio of the swarm comet would make it a large collision target for the meteoroids in the Solar System dust cloud and collisions with these particles would slowly dissipate the swarm. Also a hierarchy of particle sizes would lead to a conspicuous spreading of the swarm along its orbit over 10,000–100,000 years under the action of the Poynting–Robertson effect. Swarm comets would thus have shorter lives than icy nucleus comets—possibly too short to agree with meteor stream data.

Some Sun-grazing comets actually pass through the solar corona, and the dusty insulating layers surrounding an icy nucleus seem to be required to enable the comet to withstand the intense heat. Swarm particles would vapourise. Even though recondensation is possible, the gas content and thus cometary activity would be much reduced.

Arguments

These are some of the arguments that crop up in the debate—a debate which is not without its fair share of invective. For example, Whipple (*op. cit.*) dismisses the swarm hypothesis as 'totally unsatisfactory' and O'Dell (*The Study of Comets* part 2, NASA S.P. 393, 591; 1976) describes the swarm and nucleus models as 'the inconsistent and the unavoidable'. Lyttleton in his paper calls the icy nucleus model an 'invalid unacceptable hypothesis' to which has been added 'an elaborate set of ad hoc assumptions to try and escape the difficulties that the initial conjecture itself entails', a model which is 'ruled out at once by the principle due to Occam'. Before introducing the icy nucleus as a hypothesis 'it would be necessary to show with scientific certainty that specific cometary phenomena exist that are incapable of explanation by already available hypotheses'. In other words dust swarm out before icy nucleus in. But why? Surely hypotheses must be judged on their usefulness and not their historicity. Both can be subjected to the scientific rigour of checking and

A liquid permanent magnet?

from P. V. E. McClintock

ACCORDING to A. J. Leggett (this issue of *Nature*, page 585) there is reason to believe that the A-phase of superfluid ^3He may display ferromagnetic properties. An isolated sample of the liquid would thus be surrounded by its own spontaneously created magnetic field in much the same way as a conventional steel permanent magnet, although the physical origins of this magnetism would be rather difficult.

The onset of superfluidity (below a temperature of 0.0026 K) in liquid ^3He is associated with the atoms forming themselves into pairs, each of which can be regarded as being in many ways rather like a giant diatomic molecule, with the two atoms orbiting around each other. A bulk sample of the liquid tries to arrange itself such that the angular momentum associated with each pair lies in the same direction, known as the *l* direction (giving rise to intriguing—and, as yet, unresolved—questions about the possible existence and magnitude of an intrinsic, macroscopic angular momentum for the liquid as a whole). In the A-phase the nuclei of the atoms in a pair are orientated parallel to each other and, because each nucleus carries a feeble magnetic moment, this might seem at first sight to offer the possibility of bulk ferromagnetic properties. It is known, however, that even in a very weak magnetic field the pairs of nuclei tend to align themselves in such a way that just as many lie anti-parallel to the field as are parallel to it. One can be confident, therefore, that the liquid will not develop any intrinsic magnetic field through spontaneous ordering of the nuclei. What Leggett has done is to point to the possible existence of an entirely different mechanism in which it is the electrons of the ^3He atoms that might be able to produce magnetic effects.

It is well known that in a rotating diatomic molecule the electron shells tend to 'slip' a bit: the atoms forming the molecules do not orbit each other like rigid spheres. Thus, a little of the rotational angular momentum of a molecule gets transferred to the electrons around each of its two constituent atoms, producing electrical currents which in turn give rise to magnetic fields. For a conventional diatomic gas, however, the net magnetic field resulting from this phenomenon averages to zero because all the molecules are orientated at random relative to each other. (The effect may still be observed, though, from the broadening of nuclear magnetic resonance lines resulting from the randomly modified local magnetic field in which each nucleus finds itself.)

For $^3\text{He-A}$, on the other hand, the 'molecules' form a highly ordered system, so that the magnetic contributions of different pairs will reinforce each other, producing a macroscopic magnetic field whose direction will depend on that of the *l* vector. Leggett estimates the magnitude of this field, and he reaches the conclusion that it should be detectable in a suitably designed experiment.

If superfluid $^3\text{He-A}$ is really ferromagnetic—and, on the basis of the simple 'giant diatomic molecule' model of the pairing, this conclusion seems to be almost inescapable—there will, as Leggett points out, be a number of interesting implications. Not least is the fact that the superfluid would apparently have been found endowed with yet another form of uniqueness: as being the only ferromagnetic liquid known in nature.

P. V. E. McClintock is a member of the Department of Physics at the University of Lancaster.

improvement. To quote Leggett: 'it is not permissible to go on copying the same assumptions from one publication to the next, while simply ignoring either the contrary evidence or the lack of positive evidence'. So the adherents of the swarm hypothesis must investigate explicitly how a swarm can accelerate as well as decelerate in its orbit, how it can split up, produce outbursts, and how it can exist as a specific entity for 10^4 to 10^5 years. Those who favour the icy nucleus must again carefully investigate processes which can produce the

observed variation of coma size with heliocentric distance, the asymmetry of coma brightness, and the sometimes quickly varying spatial and temporal light condensations in it. But how can we satisfy Lyttleton?

He predicts that there is little possibility of this argument being resolved 'short of some startling optical development useable from Earth, or that the Earth should run directly into a long-period comet or a successful cometary mission has been carried out with equipment capable of finding the nucleus if it is there'. The second of these points

could, however, have been satisfied. Comets are not rare and Earth must have run into many in its lifetime. Brown and Hughes (*Nature* **268**, 521; 1977) found that a small 5×10^{10} -g comet hits Earth on average about every 2,000–4,000 years. Now swarm nuclei have cross-sectional areas about 60 times that of the Earth. An Earth-swarm impact would be equivalent to an intense and very short lived (~1 h) meteor shower. Unfortunately this would be indistinguishable from the phenomena produced by the passage of the Earth through an active new meteor stream. On the other hand, an impact between Earth and an icy nucleus comet would produce a large explosion low in the atmosphere or at ground level; Brown and Hughes proposed the Tunguska event as a strong candidate for just this type of impact. Unfortunately the surface evidence of such a cometary impact would be wiped out by erosion and the regrowth of vegetation after a relatively short time (~100 years). So we are lucky that Tunguska occurred only 70 years ago and is still evident. Older impacts have long since been erased. Needless to say, to propose that Tunguska was probably caused by a cometary impact is a far cry from regarding the Tunguska event as definite proof that comets have small dirty snowball nuclei.

I think the evidence is strongly balanced in favour of the icy nucleus model but it must always be remembered that it is only a model, not established fact. We still are far from knowing what a comet is for certain.

Gel motility

from Dennis Bray

LIKE the electron, the cell shows a different face according to how it is treated; at least, that is the conclusion to be drawn from recent papers on cell movement. Even the intuitive picture of the inside of the cell seems to depend on the techniques used. To the biochemist for example, who isolates actin and myosin from various tissues, the cytoplasm appears to be full of miniature muscles. Membrane-anchored actin filaments slide against short myosin segments and in this way generate cell extension, membrane flow and intracellular movements. This 'biochemical' view has the unique advantage that it gives immediately the molecular details of force generation, but the overall picture is very blurred. How the small contractile elements are to be distributed and coordinated within

the cell is problematic and left largely to the imagination. By contrast, the 'cytoskeletal' view—which has been engendered mainly by the use of fluorescent antisera—shows the position of the fibrillar proteins directly. Here the cell is a complex basketwork of actin cables, microtubules and 100 Å filaments, sometimes linked together in a geometrically precise fashion. The relationship of the different proteins to each other can be seen in gross terms, but so far it has not been possible to go down to molecular dimensions. Moreover, this picture has a distinctly inflexible quality and movement with such accessories calls to mind the grudging articulation of a child's construction set.

Most recently a number of investigators have, at least by implication, returned to what might be called the cytoplasmic or 'amoebic' view. They see the cytoplasm as a substance that can flow or set on demand and thus produce within the confines of the membrane the means of locomotion. The experimental basis of such work is the cell-free extract that can either move or change its consistency in a meaningful way. This was first shown to be possible in an experiment of Allen and coworkers (*Nature* **187**, 896; 1960), and a major part of the contemporary work is a direct descendant of this. As summarised by D. L. Taylor (*Cell Motility, Cold Spring Harbor* 797, 1976), the cytoplasm of free-living giant amoebae may be isolated into various test solutions where it responds according to their composition. Without free calcium the cytoplasm is immotile and lacks birefringence while the addition of as little as 7×10^{-7} μ M calcium will cause contraction. Cells ruptured in threshold concentrations of calcium will extrude cytoplasmic 'flares' very like pseudopodia without their membranes. These transitions have been related to changes occurring within the intact amoeba and, in turn, there are indications that they depend on the state of aggregation of the cytoplasmic actin. The latter feature has been shown very clearly in recent work on the slime mould *Physarum* in which, as in amoeba, the rapidly streaming cytoplasm has two distinct phases. Isenberg and Wohlfarth-Bottermann (*Cell Tiss. Res.* **173**, 495; 1976), examined the droplets obtained by puncturing the organism which are at first sol-like and then change in consistency and mechanical strength to produce a gel. The drops were allowed to spread on the surface of small aliquots of buffer and then caught on an electron-microscope grid. Under the right conditions, within 5 minutes or so, a dramatic increase

occurred in the numbers of actin filaments concomitant with the maturation of the droplet: strong evidence that actin polymerisation is involved in the sol to gel transformation.

Another lineage of experiments, arising from a different initial observation but converging to a closely analogous position, concerns motile extracts of giant amoeba, acanthamoeba, slime moulds, sea urchin eggs, macrophages, leukaemic cells and HeLa cells. These systems were recently reviewed by Hitchcock (*J. Cell Biol.* **74**, 1; 1977), to whom the interested reader is referred. These extracts resemble the isolated amoeba cytoplasm in many of their properties but since they involve a number of purification steps they are—in spirit if not in simplicity—considered as biochemical preparations. They share an initial extraction into ice-cold buffers lacking calcium and containing ATP, and most of them use either sucrose or glycerol. They all show a temperature-induced gelation and they all have as their major component actin.

Beyond this the differences are many and somewhat confusing. Compare, for example, a recent paper on *Dictyostelium* (*J. Cell Biol.* **74**, 901; 1977), with one on cultured HeLa cells (*J. Cell Biol.* **75**, 95; 1977). One uses sucrose and a detergent in the extracting solution, the other a simple buffer; one extract will contract after gelation, the other will not; only a handful of the 20 or so proteins present in the extracts are obviously comparable, and so on. The burden to the casual reader is not made lighter by the zeal with which the workers in this field publish the minutiae of their results and their papers are characterised by the number and complexity of tables of ionic effects and figures showing protein composition. If there are general conclusions to be drawn from the comparison of various systems, the authors do not make them easy to find.

This is not a trivial point because it is possible to become data-drunk and confuse the formal similarity between the various observations for a common mechanistic basis. One can finish with the belief that the patently major cytoplasmic streaming and sol-gel transformation of a free-living giant amoeba is true of a vertebrate tissue cell: it may be so; but it has not yet been shown. Most importantly, the fact that these various sources can all yield protein gels is not evidence of a common physiology. Most proteins will form a gel if they are sufficiently concentrated—including muscle F-actin left in a refrigerator for 2 weeks—and their consistency and mechanical properties will inevitably depend on temperature

and ionic composition. No doubt there are gels and gels; but techniques are not available to make subtle distinctions. The usual test of how an extract falls out of an inverted tube is no more scientific than the criterion advanced by Fats Waller: that it must be jelly 'cause jam don't shake like that!

To be fair, the investigators are aware of these problems and make every attempt to relate the phenomena they are studying to cellular physiology. Already there are encouraging signs, such as that the contractile properties of a macrophage extract depend on the phagocytic activity of the cells (Stossel & Hartwig, *J. Cell Biol.* **68**, 602; 1976), and that cytochalasin B has a pronounced effect on the properties of some gel systems (Weihsing *J. Cell Biol.* **71**, 303; 1976; Hartwig & Stossel *J. Cell Biol.* **71**, 295; 1976). One can hope that the motile gels will be further dissected and even that they can reconcile the presently diverse views of cellular locomotion. We are so spoiled by the example of striated muscle—where the whole range of movement, from macroscopic changes in length down to molecular or even submolecular changes, can be linked in an unbroken series of logical steps—that nothing less will do. □

Relevance of parasitology

from F. E. G. Cox

The British Society for Parasitology autumn meeting on The Relevance of Parasitology to Human Welfare Today was held in London on 28 October, 1977.

THERE is no doubt that parasitology has taken off, but it is germane to ask if some of it may actually have gone into orbit. It is, therefore, essential for parasitologists to get their feet back onto the ground and that is what this meeting was about. In the morning B. O. L. Duke (World Health Organisation (WHO), Geneva) spoke about medical aspects of parasitology, R. B. Griffiths (Food and Agriculture Organisation, Rome) about veterinary aspects and P. T. Haskell (Centre for Overseas Pest Research, London) about agricultural aspects. In the afternoon two invited speakers commented on each of these aspects and introduced specific and general discussion.

The morning's speakers set the stage,

F. E. G. Cox is Professor of Zoology at King's College, London.

summarising the main areas of interest to and reiterating the parasitological problems of the developing world. The WHO Special Programme (*News and Views* **262**, 85; 1976) was outlined, but there can be few parasitologists who do not know what the questions are and no real attempt was made to provide any answers. The Special Programme is arguably one of the most important events in the history of parasitology and it would be a pity if it deflected attention from parasites which are not of medical importance. Griffiths left no doubt that diseases such as fascioliasis, trypanosomiasis and theileriasis maintain a stranglehold on meat production in the tropics, and singled out ticks as the major vectors of the diseases of domesticated animals. Considerable progress has been made in the control of these diseases—new and effective anti-trematode and cestode drugs such as Praziquantel are now available and successful immunisation of cattle against east coast fever has been achieved. Less dramatically it has been shown that larval stages of tapeworms which are not infective to man can be differentiated from those that are, making it possible to save vast numbers of carcasses. It may be that the amount of meat saved as a result of parasitological research is not much by western standards, but it is a major contribution towards raising the standard of living in the developing world and supporting the increasing population which will result from WHO activities.

Agricultural improvements are also necessary for the future of the developing world and there is evidence that the problems are not insoluble although insecticides will remain the major tool for many years to come. There is, however, much more that needs to be known about tropical ecosystems before it becomes possible to modify local or devise new agricultural methods, for it is now clear that the transplantation of western ideas and techniques creates new problems as well as solving some of the existing ones. In particular, the importance of plant nematodes is only just being understood (J. Bridge, Imperial College, London), and the differences between their activities in tropical and temperate lands may be immense. Land use in the tropics, especially Africa, is a perennial topic for discussion and W. E. Ormerod (London School of Hygiene and Tropical Medicine) drew attention to the limitations that soil and rain place on land use (see also Ormerod *Science* **191**, 815; 1976). The use of indigenous animals as sources of food was discussed, but possible parasitological problems were not considered.

One of the major sources of disquiet

discussed was the lack of any real future for drugs against parasitic diseases. J. F. Ryley (ICI Ltd, Alderly Park) pointed out that the development of any drug was now prohibitively expensive, mainly because of the regulations embracing such developments. The WHO also laid down conditions which effectively prevented many pharmaceutical firms from co-operating in joint ventures. Participants at the meeting were pleased to learn that these conditions are to be changed.

Another source of disquiet was the lack of career prospects for young parasitologists. Graduate unemployment is a world-wide problem and developing countries naturally wish to employ nationals in WHO and other schemes. The disenchantment of young people who have a real interest in parasitology and its direct application but who find that they cannot work where they feel they are needed is real. G. S. Nelson (London School of Hygiene and Tropical Medicine) drew attention to the achievements of parasitologists in the past and the important discoveries made by Britons in this field. It may well be that, in the past, devoted parasitologists working in less than optimal conditions took the assistance of local staff for granted. Perhaps the great discoveries yet to come will be made by local parasitologists and young Americans and Europeans will be better employed as their highly skilled technicians. It takes courage and real devotion to do the work and receive none of the glory, but the great achievements listed by Nelson were those of men who thought more about sick Africans than the number of papers published. □

Two-photon ionisation interference effects

from Peter Knight

AN atom in a moderately intense laser field can ionise by absorbing more than one photon, and multiphoton ionisation is a very active subject at the moment. For example, it was hoped that the multiphoton ionisation of an alkali could provide, by courtesy of the spin-orbit interaction, a useful source of spin-polarised electrons. Preliminary experiments were performed at several laser wavelengths on the two-photon non-resonant ionisation of atomic caesium ($\text{Cs} + 2h\nu \rightarrow \text{Cs}^+ + e$) by Van der Wiel and

Peter Knight is Jubilee Research Fellow in the Department of Physics at Royal Holloway College, London.

Granneman (*J. Phys.* **B8**, 1617; 1975). Much to everyone's surprise large discrepancies, of up to four orders of magnitude, were found between experiment and until then currently accepted theory. A predicted deep minimum of 2.6 eV photon energy in the two-photon ionisation curve arising from cancellation of 6p and 7p intermediate state contributions is entirely absent in the experimental data. Three years later, after much theoretical and experimental work, the problem remains, with no sign of a successful explanation. Similar paradoxical situations exist in the multiphoton ionisation of other atoms.

The experimental two-photon ionisation rates, P_2 , are measured away from intermediate atomic resonances and at low enough laser intensities I to avoid saturation. Then the two-photon ionisation generalised crosssection σ_2 at laser frequency ω can be defined through $P_2 = \sigma_2 \langle I^2 \rangle$ and σ_2 is described by the second order perturbation result

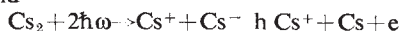
$$\sigma_2 \propto \left[\sum_n \frac{\langle f | r | n \rangle \langle n | r | g \rangle}{\omega_n - \omega} \right]^2$$

The atom is presumed to start in some initial state $|g\rangle$, end up in a continuum state $|f\rangle$ and we need to sum all intermediate states $|n\rangle$ of energy $\hbar\omega_n$. Often, as here in Cs at 2.6 eV, there is destructive interference amongst the terms in the sum: all signs are equal except energy denominators and σ_2 should exhibit a deep minimum sometimes called a Fano or Cooper minimum. Problems facing the theorist involve finding accurate (although necessarily approximate) values for atomic wavefunctions using quantum defect, model potential or other methods and then to perform the summations. The existence and properties of the minimum have been reinvestigated by Teague *et al.* (*Phys. Rev.* **A14**, 1057; 1976) again assuming the validity of second order perturbation theory. They find the position of the minimum is insensitive to the choice of wavefunctions. There is some disagreement between theoretical results obtained by different methods, but they all differ very much more from experiment than from each other.

The original experiments of Van der Wiel *et al.* were performed using the nine argon-ion laser wavelengths available, and were calibrated by normalisation to a known one-photon cross section measured using frequency doubled laser light. The quadratic intensity dependence of P_2 (and therefore the absence of saturation) was checked using neutral density filters. Since it is only a two-photon process, photon statistics of the laser light are relatively unimportant (they can contribute a factor of two at most). One problem is the contribution dimers make to the ion signal by processes such as



and



Relatively few dimers can provide a larger count rate than the numerically superior free atoms because they are resonantly enhanced and have a large density of resonant states. This molecular effect potentially can fill in the experimental minimum, but Van der Wiel and colleagues at FOM have studied these processes in detail (Granneman *et al.* *J. Phys.* **B9**, 865; 1976; Klewer *et al.* *J. Phys.* **B10**, 2809; 1977) and believe they have the dimers under control and that the discrepancy is not due to molecular background. Another possibility was that the minimum lies below the lowest energy available from an argon ion laser. This possibility seems now to have been eliminated (Klewer *et al.* *J. Phys.* **B10**, L243; 1977) by using an argon-ion pumped Rhodamine 6G dye laser to study two-photon ionisation at photon energies from 2.2 eV to near the two-photon threshold at 1.95 eV. Provided the temperature is raised sufficiently to remove dimers no detectable atomic two-photon ionisation remained in the wavelength at the (lower) intensities available from their dye laser. The data from all the wavelengths is compared with the best current theory in Fig. 1. In the same paper the ratio of the ion count rates obtained from two-photon ionisation of Cs atoms using linearly and circularly polarised light at the nine argon-ion wavelengths were measured. Again there is disagreement with theory, with the experimental ratio ($\sigma_2(\text{circ})/\sigma_2(\text{lin})$) lying significantly below the 'best' values of Teague *et al.* except close to a $7P_{1/2}$ intermediate resonance.

So we are left with a considerable problem. The approximate position of the Cooper minimum in the two-photon ionisation is determined by only a few matrix elements corresponding to transitions to nearby levels. Other levels shift the minimum by only a small amount.

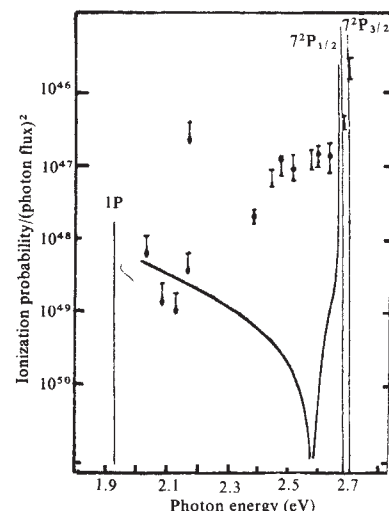


Fig. 1 Two-photon ionisation probability of Cs as a function of photon energy. **○**, Granneman and Van der Wiel (*J. Phys.* **B8**, 1617; 1975); **●**, Klewer *et al.* (*J. Phys.* **B10**, L243; 1977), $\uparrow$ upper limit found in the same ref.; full curve, Teague *et al.* (*Phys. Rev.* **A14**, 1057; 1976) theory. (Taken from Klewer *et al.*)

It would require a change in sign of either the matrix elements $\langle 6p | r | 6s \rangle$ or $\langle 7p | r | 6s \rangle$ for the deep minimum not to occur. Obviously more theoretical work on the calculation of σ_2 is needed. Even the validity of second-order perturbation theory to describe the two-photon ionisation has been investigated, by Crance and Feneuille (*Phys. Rev. A*, in the press) who studied the time development of the pulsed photoionisation using a model introduced by Armstrong, Beers and Feneuille (*Phys. Rev.* **A12**, 1903; 1975). They have found some effects due to the finite pulse length, but their model is a little idealised. More experimental work is needed and this is being undertaken in various laboratories. Looking for a minimum is hard when background molecules are only too eager to fill the sought-for trough and future experiments will need to pay special attention to this problem. $\square$



A hundred years ago

DR. VOHL, of Cologne, has adopted an ingenious method of determining the impurities in the Rhine, which consists in analysing the boiler incrustations of the river steamers, as well as the concentrated residues remaining in the boilers after passing over a certain distance. By this means he has detected the presence of a large amount of arsenious acid in the river water—resulting chiefly from the aniline and dyeing establishments—as well as other poisonous substances. An unusually

high percentage of phosphoric acid showed that the sea was daily absorbing vast quantities of the most valuable fertilising material from the soil of Germany.

ANOTHER sitting of the enlarged Council of the Observatory of Paris was held on December 9. The councilors passed a resolution for an increase of the salary of the astronomers and auxiliary astronomers, the maximum pay of the former to be 10,000 francs instead of 8,000, and of the second 7,000 instead of 6,000. They propose to the Government to place the appointment of the director of the establishment partly in the hands of the Academy of Sciences and partly in the hands of the Council, the Minister to have only the privilege to choose between both presentations. From *Nature* **17**, 13 December, 131; 1877.

THE lunar geological formations known collectively as the Fra Mauro Formation have long been thought, by most students of the Moon, to be deposits of ejecta from the basin of Mare Imbrium. When, in 1971, Apollo 14 astronauts surveyed an area not far from the crater Fra Mauro itself, it was hoped that new light would be thrown on this old conjecture.

The *US Geological Professional Paper 880*, US Geological Survey, 1977 examines merely a part of the apposite evidence: that relating, principally, to the astronauts' *in situ* findings and their hand-held camera records. Accounts of the terrain characteristics and of numerous rock blocks feature prominently in the description. The rock blocks range up to several metres in size. They are all breccias thought to have been produced by the welding of impact-generated fragments. The photogeological analyses of these rocks cover discussions of their structures and descriptions of their clasts. One block of rock has a probable clast, 1.7 m in length, in which other clasts are found and which themselves contain yet other probable, small clasts: this indicates successive and discrete stages in the evolution of this particular block.

The photointerpretation is supplemented by the astronauts' own field descriptions of the samples; and the publication includes coloured geological maps, charts, and panoramic photomosaics to assist the reader with the identification of a sample's relative position and environs.

Good descriptions are presented of three kinds of rock fillet—an accumulation of fine-grained material (see photo) making contact with a co-

Surveying the Moon

from G. Fielder

herent block or rock—and of other features which point to physical processes on the lunar surface. Among these features, the lunar grid (lineament) system is put in perspective when the authors argue for the correlation of small-scale (centimetre to metre lengths) lineament trends with trends mapped on a large scale and dismiss the proposition that lighting

can have an all-important bearing on the apparent directions of most lineaments.

Although the matrix materials of the rocks studied in detail on this fine collection of photographs are frequently referred to, in the text, as of probable Imbrium origin, the Fra Mauro Formation is said (without qualification), in the Summary of Conclusions, to consist of ejecta from the Imbrium basin! □

G. Fielder is Head of the Lunar and Planetary Unit at the University of Lancaster.



One of three kinds of lunar fillet: lunar regolith fines have been scattered on to this gently sloping, structured rock face and have settled in shallow depressions to produce a 'low angle fillet.' (NASA photo AS-14-68-9450)

Benioff splitting

from Peter J. Smith

BENIOFF ZONES are intimately associated with earthquakes. Indeed, earthquakes provide the best means of delineating such zones, indicating with startling clarity how moving oceanic lithosphere bends downwards beneath appropriate continental edges and descends at an angle of about 45° until it is ultimately assimilated into the mantle. But whereas it is customary to see a scattered pattern of earthquakes defining the outlines of a descending plate, more recent observation has indicated the presence in some

cases of a fine structure of seismicity which may throw light on at least one of the processes by which Benioff zone earthquakes are produced.

A nice example is provided by Engdahl and Scholz (*Geophys. Res. Lett.* **4**, 473; 1977), who have analysed the distribution of foci from all earthquakes with magnitude greater than 3 occurring from July 1974 through February 1977 in the Adak region of the central Aleutians. The resulting plot shows that most of the shallow seismicity in the area occurs within the depth range 15–27 km and has a lateral spread of about 50 km in the direction of the horizontal component of plate motion (that is, viewed in side projection, looking end-on at the arc). Immediately below, the zone of seismicity then contracts to a lateral width of no more than 10–15 km. At a depth

of about 100 km, however, the seismicity splits into two distinct zones each of which has a lateral dimension of 10–15 km and is separated from the other by a practically earthquake-free region about 25 km wide. At a depth of about 175 km the two arms then come together again, forming a single zone of seismicity down to the deepest earthquakes at about 270 km.

This is not the first known example of Benioff splitting. Sykes (*J. geophys. Res.* **71**, 2981; 1966) observed a separation of about 30 km in the pattern of intermediate earthquakes beneath the Kurile arc more than a decade ago; and more recently Veith (thesis, Southern Methodist University, Texas, 1974) and Stauder and Mualchin (*J. geophys. Res.* **81**, 297; 1976) have investigated the Kurile case in greater detail. Umino and Hasegawa (*Zisin* **28**,

Peter J. Smith is a Reader in the Department of Earth Sciences at the Open University.

125; 1975) have also demonstrated the existence of splitting beneath Japan. In each case the earthquakes in the upper arm of the split indicate down-dip compression whereas those in the lower arm show down-dip tension.

But is such splitting typical or atypical of Benioff zones? And either way, what causes splitting and the associated difference in focal mechanism? The answer to the second question may well suggest an answer to the first. One possible explanation is that the two arms of seismicity represent two closely associated, but distinct, descending slabs. But although this possibility is not inconsistent with the Adak splitting, it cannot, according to Engdahl and Scholz, account for the Japanese and Kurile examples; nor can it explain the difference in focal mechanisms in any of the three cases. Then it is possible, as suggested by Veith, that splitting is related to the olivine-spinel phase transition, although Engdahl and Scholz make no comment at all on that.

What they do, however, is to propose, following Isacks and Barazangi (in *Island Arcs, Deep Sea Trenches, and Back-Arc Basins*, edit. by Talwani M. & Pitman W. C., American Geophysical Union, 1977), that splitting is due to deformation of the lithosphere during subduction, and to follow up the proposal by constructing a model which seems to account for the observation. The basis of the model is the fact that subducting lithosphere bends at shallow depths but straightens out again as it moves at an angle towards the mantle. Whatever the cause of these changes, the 'unbending' must produce stresses in the descending slab; and Engdahl and Scholz are able to show with little difficulty that, at the observed depths of splitting, an 80-km thick lithosphere has a 22 km thick elastic core with down-dip compression on its upper surface and down-dip tension on its lower surface.

Thus the double zone of seismicity can be explained simply as the result of the release of unbending stresses above and below an elastic core within the lithospheric plate. Moreover, as the plate descends into hotter and hotter regions the elastic core will get thinner and ultimately disappear altogether, which explains why splitting occurs only over a limited depth range below the initial bending of the lithosphere and does not persist to all depths. Since unbending must occur in most, if not all, descending plates, it follows that splitting must be typical of Benioff zones. Failure to observe it in any given case presumably therefore reflects only insufficiently detailed observation and analysis of earthquake foci. □

Jeffreys Lecturer links Sun and weather

from John Gribbin

In this year's Harold Jeffreys Lecture of the Royal Astronomical Society, presented on November 11, J. W. King of the Appleton Laboratory reported the latest work of his team on 'The Influence of Solar Phenomena on Weather and Climate.' Over the past few years, King and his colleagues have received a rough ride from some quarters for their failure to explain the physical basis of the links they have found between Sun and weather; commenting here that the situation is no different from that in climatology, where we know the Earth's climate does change without being able to say exactly why it changes, King went on to present convincing evidence of the reality of the link on timescales down to days, and threw out the challenge to both solar physicists and meteorologists to join the Appleton team in investigating these phenomena further.

To most of the audience, and most readers of *Nature*, the debate about solar influences on weather over the sunspot cycle and longer periods must already be familiar. But the new work from the Appleton Laboratory concentrates instead on much shorter term influences, associated with the roughly 27.5 d rotation period of the Sun. The 'signature' of this rotation shows up clearly in such meteorological parameters as the height of the 500 mbar pressure level in the atmosphere, as well as influencing ionospheric properties. In some danger of numbing his audience with overkill at times, King hammered home the reality of these links with a wealth of data, the most intriguing of which showed clear geographical variations of the magnitude and even sign of the atmospheric changes produced by the Sun. Small wonder, then, that global averages over long periods show much less indication of any solar influence on weather!

The solar influences are particularly strong in a region above the Atlantic just west of the British Isles, which makes this link between Sun and weather especially important for residents of those islands. And if these small variations over the solar rotation cycle can affect weather parameters, then it comes as no surprise to find specific larger events on the Sun—flares and so on—producing specific larger disturbances of the Earth's atmospheric systems.

Such a link has, in fact, been known for some 30 years, since the pioneering work of Duell and Duell (*Smithsonian Misc. Coll.* 110, 8; 1948) but has only relatively recently become firmly

established (see, for example, Olson *Nature* 257, 113; 1975). The Appleton team have now found that not only does a solar flare produce a disturbance of the atmosphere, commencing some four days after the flare, as shown by the work of Olson and others, but that the atmospheric disturbance is repeated at 32 and 60 days as well, one and two solar rotations later. In at least one case, an effect on the atmosphere is also found at -24 d, that is one solar rotation before the flare became apparent. So a specific region of the Sun which is involved in some disturbance can affect specific regions of the Earth's atmosphere over a period of 2-3 months—and, equally, the effects of solar activity which are disturbing the atmosphere today must be the integrated effects of several such past disturbances, which suggests that we should not find any simple relation between what the Sun is doing today and what the atmosphere does tomorrow.

King also described investigations of the longer run of data, going back for 100 years, available from UK Met. Office statistics, and these show a clear influence of the solar activity on occurrence of westerly weather patterns over the UK, that is, the weather coming from the region of the Atlantic where the solar influence is strong, and moving towards the British Isles. But this evidence was merely the icing on the cake of his presentation.

The challenge to meteorologists is clear, since these effects are certainly large enough that by ignoring them all the present general circulation computer models (GCMs) must be in error. Could this be why such models are notoriously unreliable if run for more than a few days ahead? And equally the challenge is there for the solar physicists to explain what is going on—with the incentive that the work becomes of direct importance and value now that the link with the weather is emerging.

So King closed by saying that the pathfinding stage of this Appleton Laboratory work is now at an end, with the establishment of the reality of the Sun-weather links. In this essentially interdisciplinary study, the time has come for collaboration between the Appleton group at the centre and the meteorologists and solar physicists on either side; 'I hope,' he said, 'that this lecture will mark a starting point in progress towards achieving practical benefits.' □

John Gribbin works at the Science Policy Research Unit at the University of Sussex.

My Uncle Eustace—Maker of Rings (1885–1916)

A HAPPY solitary child, after
his mother died so self contained
and yet attached by magnet
chain to Margaret the country girl
who mothered him at Vicarage;
his father never understood
or tried to peel, excise
the layers of his mind, beneath
the courtesy, the grave concern
so strange in one so young, one sensed
always a sunlit glow, a pool
of quiet stillness that no naval mine,
no six-inch shell could shatter.

He passed, as far as clerical
finances would permit, to prep., then
boarding school, no niche
seemed ready carved, a solitary boy
puzzling to House and Form
master and sixth form prefects, yet
always just scraping through
two from the bottom everywhere,
cold shouldered, taunted, ragged
he would endure quite unconcernedly, until
one famous bully, older by two years
provoked him ceaselessly and on one afternoon
when batting on the Common, suddenly
young Eustace threw down his bat and strolled
slowly down pitch and felled
braggart to turf with one fierce swing.
Always, when this deed had sunk
swiftly to groundswell, not merely left
in peace but elevated to that slot reserved
for the eccentric, rare, unique, original.

One confidant he found, old Bony Head
the science master who could sense
a mind attuned intuitively to select,
assess causal relationships, communicating flows
built up from pulsing waves in orbit,
sure, symmetrical, cast in one mould, one unitary mass.

Astounded and affronted was his family,
that hierarchy of patrons, chiefs,
that network with edges pinned in Burma,
Delhi, London when he gained
a science scholarship to Trinity,
a field, a slippery slope fit
for no gentleman to tread and yet,
with mother's tiny nest egg to augment,
how could he be gainsayed?

Sheep-like he merged within the Cambridge
landscape; who knew or cared
exactly where his lodgings lay
or whether he built up
stable relationships? He never said
how marvellous those days, how glorious the golden youths
with whom he melted smoothly down in Hall
or rowed in college eight.
Always and only shone an inner light.

His tutor was non-plussed because
over the wide field of chemistry, he barely
could pass muster, might just expect
to scrape a Third and yet in one small
unexploited corner of the craft he delved
and bored and quarried ceaselessly;
the hetero-cyclic, alicyclic rings,
those compounds linked from carbon, nitrogen,
oxygen, phosphorus and halides, elements
rare, of dubious and unestablished
property and mass and controversial valency
and metals of odd groups, forced them to bind,
adhere in hoops and circles, cubes
and boats and knotted cord-like
thongs; these problems he devoured,
consumed with every meal, by them
he was possessed, their literature he absorbed
in tiniest detail, their personalities,
their history; as then there was no other specialist
at hand, this bred a bright hot fury of debate
whether he merited a First, a Third, a Pass.
Faith won the day, though outwardly indifferent,
or so he claimed, so long as he could scoop
a bursary to study for two years at Heidelberg,
the fountainhead and spring of all clear waters
here in this smooth fluency in which
his flimsy ship was launched, must sail
and pitch and toss on ocean till it sank
shipwrecked on rocks, or ran relentlessly
to calm deep water harbour.

But Eustace never felt at home with Germans,
yet from them learnt all that he could,
kept secret much, ploughed down to dusk
and thirty days a month, except
for Sundays when he'd read and walk
to early service in the English church;
how, in those days, these days, those ever
other days can one combine science
with Christ? Well, Eustace tried and yet
he'd never talk, explain, attempt to justify,
but said 'it's part of me and always will be,
so please don't push me further'. How much
privat-docents, professors knew
or cared or understood about his bench-work
problematical. Much that he wrestled with
he never spoke about, he sealed his notes
tight locked within a safe. After two years
his thesis was submitted, was so unique,
outside all normal channels, unbelievable
until one sub-professor delegated for two months
repeated parts in the minutest detail
and found each component and experiment
immaculate, repeatable with all analyses
confirmed in unimpeachable statistical
integrity; in essence so non-plussed
the academical establishment, so much at doubt,
so acrimonious the conflict that at last
his D.Phil. was awarded and they asked
that he submit his name for hierarchical preferment.
Eustace said 'NO' and coming home in nineteen
ten found no response from British
chemical grandees in any university.
Instead, sensing in bone that school

or industry demanded volumes of life-blood he dared not lose and live, would dislocate all plans for studies targetted, he ferreted instead a newly mortared niche in red brick college of technology in Yorkshire. Here, simply was demanded three lectures weekly and all the space of evenings, week-ends and Long Vacations free to theorise, experiment and scribble round the clock.

All work, you say, no private life and so it might have been had he been able to forget those German years; within his blood-stream multiplied the germs of fear, of certainty of wars to come. Exactly numbered days and calibrated therms of energy he gave to Territorial Brigade of Heavy Artillery, became an adept at range-finding, the most professional of officers from whom Colonels of regulars bent down to claim advice. Upon parade he stood one rank beneath my father, who, since a boy-hood thirled to the Lovat Scouts was always army mad. I never knew another so assiduous at work, at drill. He breathed in Elgin ice clear air where grandfather was bank cashier but dad determined from the very day he learnt to read to mount two ladder rungs beyond his family, became Chartered Accountant, then set up in solitary practice here in Yorkshire, where his twin Fiona kept house for him and taught German and English in Ladies' Private School.

Enmeshed by such a pair, how could poor Eustace disentangle from the cords of fishing net? My Aunt Fiona was not outwardly clever at all, a quiet fair haired filly, she seldom spoke so that the Head judged her to be submissive, compliant and then finances being strained, conveniently forgot Fiona's name on the Promotion List and when her resignation came by the first post next morning, a Parents' Protest Committee quickly crystallised and threatened to remove their girls to local Grammar. So, my uncle equally became submissive, really in fact enjoyed his thralldom, as she controlled all family affairs and left his week-ends free for pure research and gunnery.

A scientist is judged by all he publishes, the volume and the quality, the range of the discoveries and theories that appear in journals of accredited repute, the details of the fields that he has ploughed, the implements and measures at the base of tower and palace and with what ease his syntheses were found repeatable and could be used as spring board for fresh thinking. As my uncle Eustace published *nothing*, his reputation faded by neglect and he was soon forgotten by the élite of academic chemistry.

One Sunday, after tea, both families still sitting round the cloth, we children, cousins, were dismissed to garden. Rain, lightning shattered down and we returned to the worse thunder of a family dispute. 'To Hell', my father shouted, 'with academic honour. "May science never be any damn use to anyone" is fine for younger sons of viscounts but not for you, young Eustace, you don't possess a bean and when war comes, what will you leave your wife and kids to live on, if we're killed, as very well we may be; why should they starve because you are too proud to patent, publish your really rare discoveries? Now, fetch them out this minute, there's a good lad and we'll discuss their possibilities.' Fiona sided, not with my father but with her husband, swore she'd work and could support them all. But Uncle Eustace, in sudden silence, strode to his study and thence bore back a pile of manuscripts. While we all watched, in silent awe, the two men there and then selected ten of his theses which my father slipped away, for processing by patent agent friend.

When nineteen fourteen broke, the Battery with other amateurs was very soon immersed in battle. For two whole years my uncle strove with howitzers and mortars. His easy skill soon earned promotion and he found himself a temporary major at Army Corps Headquarters but, odd man out as usual, this election served only to convey boredom, so he transferred to Flying Corps and brought to bear his ingenuity in coaxing speed, manoeuvrability, quick reflex action from his Sopwith Scouts, high above battlefields in dog fight and reconnaissance.

Your past will find you out, advance upon you and on one August afternoon, recalled to C.O.'s hut he was commanded to collect all gear as he was ordered back to Ministry in Whitehall where the back-room boys had delved among his patents and discovered there the basis for a new, intensely powerful explosive which might win the very war. My uncle, angry, overruled, strolled slowly across airfield to his plane, to bid good-bye, as to a favourite terrier dog and at that hour the German fliers dived from cloud to rake the 'drome with fierce machine gun fire. Thus, Uncle Eustace died.

A posthumous hero, a genius accorded after a death so quizzical, so individual, yet so much of the pattern he had set himself to follow: my thoughts, my words still after thirty years confused, ceaseless in motion change from day to day and still I cannot say why this should be or if a yet more glorious world might shine had Uncle Eustace lived, lined up with others of his tribe, with Moseley and Wilfrid Owen, Rupert Brooke, the roll is endless.

I can record twenty-five monographs in Transactions of Academies world-wide, his name on plaques, on chapel walls, columns and references in text books, scores of new compounds indexed, in this his family name. Even the Fifth Form schoolboy fails if he forgets what Eustace stands for, and, as for his patents, Dad was very soon besieged by major companies in Widnes, Delaware, Paris, Turin and Hamburg outbidding all to purchase royalties for synthesis of basic chemicals for fertilisers, textiles, dyestuffs, drugs. So Father waxed, became commander on a field he was well qualified to master, transferred his offices to London, was made director of ten companies, Lord Mayor and baronet.

Succeeding him, I feel
a conflict of emotions; undeserving, why
should I and all my family be circled round
with so much satiety, delight, so many friends
and joys of love and freedom, beauty, esteem and honour
and all because of one young uncle who
pursued his goals with such humility
and silence and a quiet smile? And yet
I never can forget that glow, that steady
luminescence in his eye which I, as child
divined and marvelled at, sitting without movement
beside him on the window seat, those birds,
those flowers, as are the stars unquenchable,
eternal, so can I never understand
how light of such a clarity can fade,
what medium that I can comprehend
could generate the power
to drown and snuff it out?

Deric Bolton*

Deric Bolton was, until 1971, a chemist with Macfarlan Smith Ltd, Edinburgh. His four published collections of poetry include The Wild Uncharted Country (1973), a narrative sequence on life in the chemical industry.

**17, Wester Coates Avenue, Edinburgh 12, UK.*

Three centuries of alcohol in the British diet

Josephine A. Spring & David H. Buss

Nutrition Section, Ministry of Agriculture, Fisheries and Food, London, UK

Alcoholic drinks were consumed in larger quantities in the eighteenth and nineteenth centuries than in the twentieth century, although there has been a recent increase from the historical low of 1930–60. Beer, spirits and wines once provided at least 2 MJ (nearly 500 kcal) per person per day compared with 0.67 MJ (160 kcal) in 1975, towards an average energy requirement of the total population little different from that needed now. Beer has always contributed most to the alcohol, energy and nutrient content of the diet, although its importance relative to spirits and wine has declined.

THERE is much concern about the amounts of alcohol drunk in the United Kingdom^{1,2}. It is believed that men and more particularly women and children are drinking more, resulting in increased prevalence of drunkenness³, driving under the influence of alcohol^{1,3}, alcoholism⁴, liver cirrhosis⁴, and absence from work owing to excessive intake of alcohol⁵. It is, however, very difficult to obtain accurate information from individuals. Most, when asked, are likely to underestimate their consumption, while some, wishing to impress the interviewer, exaggerate their capacities.

A more objective assessment of overall trends can be obtained from HM Customs and Excise Department, which records, for tax purposes, the total amounts of beer, spirits and wines retained for consumption within the United Kingdom (except for the small amounts of beer and wine made in the home). Since 1955 the Ministry of Agriculture, Fisheries and Food has assessed the energy content of these drinks and compared them with the energy content of the national food supply⁶. The results are summarised in Table 1.

We wished to place these figures into historical perspective, for this rise in the consumption of alcoholic beverages started

from an historical low point. Customs and Excise records, with brief exceptions, have been collected since 1684 and are summarised in Fig. 1. Note that this figure is an average for the total population of the country, including young children who nowadays consume little or no alcohol. A discussion of separate trends for each beverage will be followed by a general discussion of the major factors which can affect consumption. Tables 2 and 3 list some of these.

Beer

Ale has been drunk in England since Celtic times⁷ and hopped beer since the fifteenth century, but it was not until 1643 that a beer duty was imposed to raise money for the Civil War. The earliest national records of consumption date from 1684, when the beerhouse was the centre of the working man's life. Many workers were paid at public houses, which also served as labour exchanges; if a man was unemployed he could receive credit if he was a regular customer⁸. Inns also served as centres of transport, courts and even small prisons, such as the White Lyon at Southwark⁹. Entertainments such as cock fighting, bear baiting and prize fighting also took place in them. In the late seventeenth and early eighteenth centuries it was wiser to drink beer than the usually polluted drinking water. For example, Nottingham's water supply was drawn from the River Leen which also served as the main sewer of the town; fortunately Nottingham had one alehouse for every 80 inhabitants⁹.

Beer consumption reached a maximum of 832 pints per person per yr (or 2.3 pints per person per d) in 1689. Consumption dropped sharply when the duty was tripled (see Table 2) with partial recoveries after 1700 and 1712 when part of the tax was transferred to the constituent malt. Consumption then declined steadily until 1790 while the duty remained relatively static (as did the price: in London porter remained at 3 pence per quart until 1761 and 3½ pence until 1799 when it was increased to 4 pence⁹). There are no obvious reasons why consumption

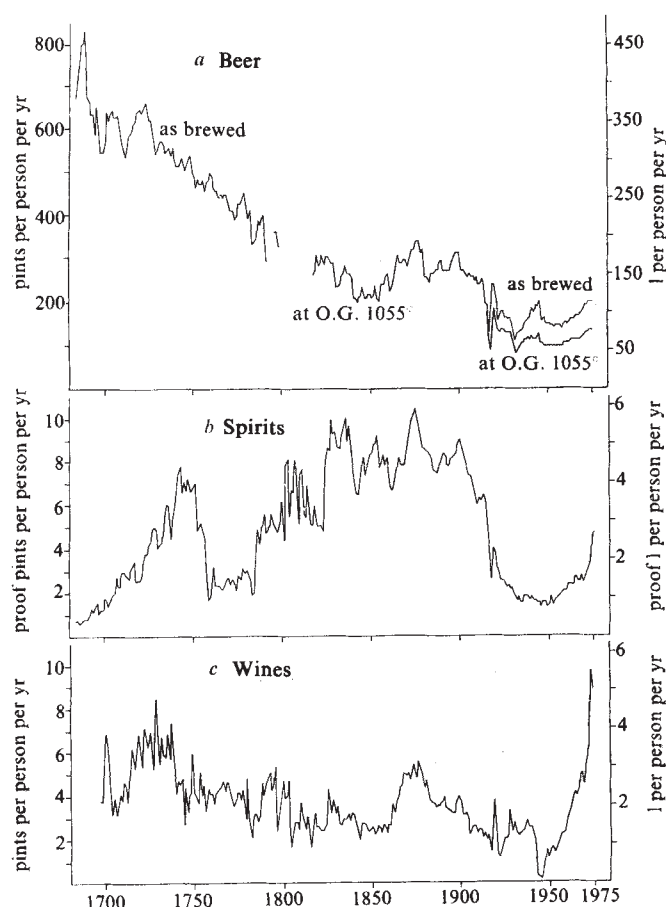


Fig. 1 Beer, spirits and wine available per person in the United Kingdom. *a*, Production figures for beer represent barrelage brewed by common brewers and brewing victuallers, corrected until 1830 to include private brewing. From 1684 to 1830 they are taken from Monckton²³, from 1831 to 1936 from Wilson¹¹ (appendix F) and from 1937 to 1975 from the Annual Reports of HM Customs and Excise Department²⁴. Volumes and populations refer to England, Scotland, Wales and Ireland up to 1922; afterwards Eire has been excluded. *b*, Values for spirits from 1684 to 1799 are from the Department of Inland Revenue²⁴, from 1800 to 1935 from Wilson¹¹ and from 1936 to 1975 from the Brewers Statistical Handbook³. Volumes and populations refer to England only until 1713, and between 1718 and 1724 to England and Ireland. Thereafter they are for the United Kingdom. *c*, Values for wines from 1697 to 1900 are taken from Wilson¹¹ and from 1901 to 1975 from HM Customs and Excise²⁴. Until 1787 they refer to wines imported into England, but subsequently are the amounts retained for home consumption in the whole United Kingdom (including Eire until 1922). The populations of the appropriate regions of the United Kingdom for decennial periods from 1684 to 1831 are from the Department of Inland Revenue²⁵ and thereafter from the Registrars General¹⁴.

decreased during this period, although from 1750 the enclosure of agricultural land and the initiation of the industrial revolution (which shifted population to the manufacturing towns) must have reduced home brewing¹⁰ particularly in the North and Midlands. In the South shortages of fuel had a similar effect⁷. In 1791 a tripling of the malt duty resulted in another sharp fall in consumption. Records from here until 1880 are incomplete, for almost all those provided by the Brewing Victuallers' Association between 1791 and 1818 are lost and those between 1831 and 1880 have been derived from a mathematical conversion of malt to beer¹¹, because beer itself was not taxed during this period, only malt and hops, and, from 1847, sugar. Consumption apparently increased as a result of the Beer House Act 1830 which allowed anyone who bought a 2-guinea licence to sell beer; 40,000 new public houses were opened within 5 yr (ref. 11).

In 1880 Gladstone introduced his free mash tun system allowing brewers to use carbohydrate sources other than malt, the duty being charged on the original gravity of the beer. This led to a decrease in price which, coupled with the nation's prosperity, caused consumption to rise to nearly 1 pint per person per d in the latter part of the nineteenth century. Thereafter consumption fell again, accelerated by an increase in duty in 1900, and reached its lowest recorded level in 1918. During World War I opening hours were restricted (see Table 3), and in 1917 production was reduced from 26 million to 10 million standard barrels and the gravity of the beer greatly reduced. The weakness and expense (after several increases in duty) resulted in a boycott of beer in many places. After the war consumption recovered, but then fell with the economic depression of the 1930s. World War II prompted a substantial increase in consumption, mainly because of the unavailability of spirits and wines, but demand was met by a reduction in gravity. Despite increases in duty, consumption remained stable after the war and rose from the late 1950s. Nevertheless the amount of beer available in the 1970s is considerably lower than it was 100 years ago.

A recent estimate put the amount of home-brewed beer produced from kits at 1.75 pints per person per yr.

Tax has been levied on cider for a few short periods only, and consumption is thus known for relatively few years. From 1756 to 1765 it ranged between 2.5 and 5 pints per person per yr; in 1820 and 1830 it was 2 pints but declined to little over 0.5 pints in some of the intervening years; and it enjoyed a brief increase to 4 pints at the end of World War I. The Cider Manufacturers Association have calculated cider consumption since 1946 as about 3 pints per person per yr until 1965, after which it rose rapidly to 5.8 pints in 1975³. Cider has been taxed since that year.

Spirits

Before the Restoration the consumption of spirits was small, but after 1660 consumption, mainly of brandy, increased⁷. In 1688 William of Orange became king and encouraged the production of gin by issuing charters to divert the surplus English grain for its production; in 1690 imports of foreign spirits were prohibited. In Queen Anne's reign the monopoly privileges of the Worshipful Company of Distillers were cancelled; this led to the unlimited production of gin, much of poor quality, which was sold in the streets and hawked from door to door at 1 penny per pint. The steady increase in consumption was checked in 1729 by the introduction of retailer's licences costing £20 per yr and the prohibition of street hawking. Distillers retaliated with a flavourless, low quality spirit, not classed as gin (known as Parliamentary brandy). In 1733 the Act was repealed, and another introduced which limited the sale of spirits outside dwelling houses. This led to the development of the 'gin shops' typified in Hogarth's engraving 'Gin Lane'¹². Thus gin and other crude spirits were both cheap and readily available at a

Table 1 Increasing contribution of alcoholic drinks to the diet

Year	Energy from food MJ (kcal)	Beer (pints)	Spirits (proof pints)	Wine (pints)	Energy from alcoholic drinks (% of food energy)
1955	13.3 (3,170)	140.2	1.8	2.7	3.0
1958	13.3 (3,180)	138.2	2.0	3.2	3.0
1961	13.2 (3,150)	155.8	2.4	4.3	3.5
1964	13.2 (3,160)	160.3	2.7	5.5	3.7
1967	12.9 (3,080)	165.6	2.6	6.1	3.9
1970	13.0 (3,110)	178.6	2.8	6.6	4.1
1973	12.2 (3,040)	196.9	4.3	11.2	5.0
1976	12.3 (2,930)	209.0	5.1	12.0	5.7

The energy content of the food supplies relates to the total quantities available in the UK from agriculture and imports, less exports and non-food uses, and is expressed per person per d. The quantities of each alcoholic drink (per person per yr) are derived from Customs and Excise records, and their energy content includes any carbohydrate as well as the alcohol present. 1 litre = 1.76 pints.

Table 2 Major changes in duties charged on alcoholic drinks

Beer and constituents			Spirits	Imported wines	Index of wholesale prices ²⁴⁻²⁷ (1860 = 100)		
1643	Small beer 6d; strong beer 2/-	1684	2d-4d	1787	French 2/3d-3/1d	1680	55
1690	Small beer 1/6d; strong beer 6/6d	1751	1/- - 1/3d		Rhenish 3/1d	1700	80
1710	Small beer 1/4d; strong beer 5/-;	1783	5/10½d		Spanish 2/-	1750	70
	malt 6½d	1785	2/7½d		Portuguese 1/6d	1800	125
1791	Malt 1/7½d	1795	4/4½d		Hungarian 3/6d	1830	95
1831	Beer duty repealed	1825	7/-		French 7/2½d	1850	85
	malt 2/7d; hops 2d	1860	10/-	1825	Rhenish	1880	180
1850	Sugar 1/4d	1890	10/6d		Spanish	1900	135
1880	Beer duty reimposed 6/3d	1909	14/6d		Portuguese	1910	160
1900	Beer 7/9d	1918	30/-		Cape 2/5d	1920	315
1916	Beer 25/-	1919	50/-	1849	Colonial 2/9d	1930	220
1918	Beer 50/-	1920	72/6d	1860	Foreign 3/-	1940	265
1920	Beer 100/-	1943	154/-	1861	All light 1/9d; heavy 2/5d	1950	475
1931	Beer 134/-	1948	210/-	1920	Light 2/6d; heavy 6/-	1960	645
1948	Beer 178/-	1964	236/-	1927	Colonial light 2/-; heavy 4/-	1970	820
1959	Beer 110/-	1968	376/-		Foreign light 3/-; heavy 8/-		
1966	Beer 188/-	1973	308/-	1948	All light 25/-; heavy 50/-		
1973	Beer 138/-	1974	340/-	1958	Light 13/-; heavy 38/-		
1974	Beer 186/-	1976	492/-	1969	Light 32/2d; heavy 54/2d		
1975	Beer 273/-			1973	Light 17/7d; heavy 39/6d		
1976	Beer 317/-			1975	Light 53/6d; heavy 70/-		

12d (pence) = 1/- (shilling) = 5p (new pence). Beer duty is per barrel (163.6 l), malt and hops per bushel (36.4 l) and sugar per hundred-weight (50.8 kg). Spirits duty is per proof gallon, that is per gallon (4.5 l) with strength corrected to 100° proof or 49.276% alcohol by weight. Wine duty is per gallon.

time when the social conditions of the urban poor were extremely bad with little hope of escape other than intoxication¹³.

The Acts of 1751 and 1752 (Table 3) resulted in a dramatic decline in consumption and for the rest of the century consumption largely reflected impositions of duty.

The wild fluctuations of the next 20 yr may be partly due to a breakdown in the system by which spirits were taxed. Indeed it was not until 1824 that the figures of the Excise Department approximate to the real production¹⁴, and all estimates of production before this date must be regarded as conservative because of the amount of illicit distilling which was practised, particularly in Ireland and Scotland. Thereafter consumption was again influenced by duty and legislation, and religious opinion had further effects: for example Father Matthew's temperance mission in Ireland caused consumption there to drop by 23% between 1839 and 1845¹¹. By the early 1870s, however, the equivalent of 4 ounces of proof spirit was consumed per week for every man, woman and child in the country; this was a time of great prosperity in the United Kingdom. Thereafter duty increases and trade depressions led to a steady decline.

In World War I, opening hours were curtailed, and the manufacture of spirits was then reduced on the orders of the food controller in 1917 to half that produced in 1916¹¹. In 1918 consumption rose dramatically with the post-war celebrations in spite of increased duties, but then declined right through the economic depression and on into World War II when available grain was used for food rather than distillation. Consumption recovered after World War II despite increases in duty in 1947, until in 1964 a substantial increase in duty led to a stable consumption rate. In 1973 the duty was decreased and the increase in consumption has now continued in spite of several more tax increases.

Wines

In the Middle Ages wine drinking was common in Britain and consumption was relatively high. The dissolution of the monasteries led to the disappearance of the British vineyards⁷. As a result, the pattern of consumption has since been largely determined by treaties, wars and tariff levels.

In 1703 the Methuen Treaty between England and Portugal allowed the import of Portuguese wine at a fixed and relatively low duty, in return for concessions on the exports of woollen cloth to Portugal¹¹. This treaty remained in force until 1831 and

was the principal reason why the British drank port during the eighteenth century rather than claret as before. Between 1704 and 1785 the proportions of wine, by country of origin, which were consumed in Britain were Portuguese 65.4%, Spanish 29.3% and French 3.6%¹¹. Consumption of port and other fortified wines was largely confined to the richer members of the community: for example one Dr John Campbell drank 13 bottles at one sitting and many men habitually drank 4 bottles of port a day¹³.

In 1783 a new treaty with France lowered the duties on French wine. This was partly designed to combat the smuggling of French wines, which was rife, and it led to an increase in consumption in the 1780s. This continued into the next century despite the Napoleonic wars and was followed by a decline in consumption in the post-war depression until 1825 when the wine duties were altered¹¹. Consumption remained steady until 1860 when the Cobden Treaty was signed with France, and the wine duty was reduced. This was followed in 1861 by the duties charged being based on the alcoholic strength of the wine; a lower rate was levied on 'light' wines not exceeding 20° proof

Table 3 Major statutes affecting consumption

Date	Statute	Effect
1751	An Act . . . for more effectually restraining the retailing of distilled spirituous liquors	Greatly increased duty on spirits
1752	The Disorderly Houses Act	Controlled excessive numbers of public houses
1828	The Alehouses Act	Restricted opening hours
1830	The Beerhouse Act	Allowed beer sales on licensed premises only
1848	The Alehouses and Beer-houses Act	Restricted opening hours
1854	The Sunday Beer Act	Reduced opening hours on Sundays
1872	The Licensing Act	Restricted numbers of licences
1908	Children's Act	Children under 14 not allowed to enter bars
1914	The Defence of the Realm Act	Public houses closed at 11 p.m. weekdays, 10.30 p.m. Sundays
1923	An Act (on) the sale of intoxicating liquor to persons between 14 and 18 years of age	Illegal to sell intoxicating liquor to anyone under 18
1945	The Licensing Planning Act	Controlled redevelopment of public houses in bombed areas

spirit. Consumption increased during the mid-Victorian period of prosperity, but declined after 1875 because of an economic depression. Consumption of port increased despite the higher rates of duty on fortified wines, but later declined perhaps because after-dinner smoking became fashionable instead. Sherry consumption increased until 1873, when the demand was so great that poor quality wine was sold to meet this demand and led to consumer resistance¹¹.

Apart from a small peak at 1900, consumption continued to decline up to and during World War I. During the war years wine consumption was not affected as much as beer or spirits: although imports of wine were checked and formal entertaining was curtailed, consumption was stimulated by the low-gravity beer and shortage of spirits¹¹. Post-war celebrations doubled the consumption in 1919, but this was reversed by an increase in duty in 1920. Consumption recovered a little in the 1930s but almost no wine was available during World War II because of the shipping blockade and the fact that many of the wine-producing countries were controlled by Axis powers.

Consumption rapidly recovered after the War, encouraged by a variety of stimuli. A reduction of duty in 1973 caused a further steep increase in consumption; the increases in duty in 1976 and 1977 seem to have checked this.

British wines and mead

Little is known about consumption of British wines before 1927 because this wine was taxed only for a few short periods. Consumption rose from 0.5 pints in 1930 to nearly 1.2 pints per person per yr in 1939, only to fall to 0.5 pints again during World War II. Only in 1960 were pre-war levels reached again. This upward trend has continued and reached 2 pints in 1975. A recent estimate shows that home wine making from kits provides 0.33 extra pints per person per yr.

Factors affecting consumption

The consumption of alcohol depends on four factors: price, availability, current social pressures, and educational or moral campaigns¹¹. Economic factors seem to be of paramount importance, as the fluctuations in the consumption of alcohol closely follow the rise and fall in the general trade of the country. Years of prosperity such as 1875 and 1899 were years of much drunkenness¹¹, and this is illustrated by Booth's evidence from a London policeman during the 1880's: "a great amount of drunkenness is still a sure sign of work being plentiful. It is then that the police are busiest"¹⁵. Equally the economic depressions of 1855–60, 1875–80, 1894, 1904, 1909 and 1928–35 were years of modest consumption. Furthermore, as we have seen, consumption is sensitive to purchase price. The prices of beer, spirits and wines relative to each other, and relative to other beverages and to food and other goods in general, also affect consumption. Coffee was introduced to England in 1637 and tea and cocoa followed around 1650⁷. They were luxuries at first and drunk only by the wealthy, but by 1800 the price had fallen far enough to make their use universal. Tea consumption rose from 1.4 pounds per person per yr in the first decade of the nineteenth century to 5.7 pounds in the last decade (and is now 7.7 pounds). Similarly coffee consumption increased from 1.1 ounces per person per yr in 1801 to 16 ounces in 1880, but then declined. (It has recently reached about 80 ounces.) Cocoa imports grew from 0.5 ounce per person in 1822 to 10.4 ounces per person in 1894. And between 1841 and 1882 taxes on tea, sugar, coffee and corn were lowered from £15.8 million to £4.8 million, while those on beer, spirits, wine and tobacco rose from £18.1 million to £37.3 million¹⁶; this helped tea to displace beer as the main drink of the poor. The price of alcoholic beverages relative to other food has also decreased recently, and it has been estimated that the number of minutes work required from a male manual worker to pay for a large loaf (1½ pounds), 1 pint of beer and a bottle of whisky have changed from 9, 23 and 659 respectively in 1950 to 11, 12 and 209 in 1976¹⁷.

Table 4 Decreasing contribution of alcoholic drinks to nutrient intakes (mg per person per d)

	Iron	Calcium	Magnesium	Riboflavin	Nicotinic acid
1691	0.27	150	133	0.83	15.7
1741	0.20	110	97	0.61	11.5
1791	0.14	67	59	0.37	7.0
1841	0.09	47	41	0.26	4.9
1891	0.12	60	54	0.38	6.3
1941	0.05	27	24	0.15	2.8
1971	0.10	29	25	0.10	1.5
Recommended intake*	10.0	500	300	1.7	18.0

Iron was calculated for both wines and beer, and the remaining nutrients for beer alone as any contribution from wines or spirits was insignificant. For derivation of nutrient content of earlier liquors, see text.

*Recommended intake for a moderately active male aged 18–35yr¹⁸. The recommendation for magnesium comes from the Canadian recommended daily intakes²⁸.

Licensing hours control consumption of all drinks on licensed premises, though any person aged 18 or over may purchase as much alcohol as he wishes to consume at home.

Social factors which affect consumption are of two types. Those which tend to decrease consumption include the social unacceptability of being drunk, the laws regarding drinking and driving and counter-attractions such as the availability of non-alcoholic refreshments, the acquisition of cheap consumer goods, smoking, gambling, thrift, travel, the cinema, radio and television which competed with alcohol for an individual's spending money. On the other side of the coin concern has been expressed about social conventions which lead to higher consumption in certain occupations such as business executives⁵; this is, however, a convention of long standing as Booth remarks on its prevalence in the 1880s¹⁵. Publicity has also been given to the so-called 'lace-curtain drinking syndrome' among those married women who can afford it; this too is not a new problem as Booth relates "All round London are growing up suburbs of small houses whose occupants have just enough to live on comfortably. Women are left at home, small ailments, the immediate stimulus of drink, that is how it begins"¹⁵. Booth also related the increase in alcohol consumption in women in the 1880s to their emancipation and increasing financial independence from men. In recent years the sale of alcohol in supermarkets, and liquor trade advertisements equating alcohol with glamorous living, may have tended to increase consumption.

The temperance movement has been the most prominent moral and educational force to affect consumption. The Victorian middle classes were horrified by drunkenness and especially by its increase amongst the working classes as they became more prosperous. Although their ancestors had accepted excessive consumption without question, they began to denounce it as an unmitigated evil. Previous social reformers had denounced spirits but had recognised beer as a cheap and wholesome drink greatly preferable to impure water, but from the 1850s temperance reformers zealously condemned all alcoholic beverages and achieved much in the imposition of restrictions on the sale of drink to children (see Table 3). The temperance movement was a formidable demonstration of the power of organised middle-class opinion and of the 'non-conformist conscience'¹⁸.

Contributions to diet

The relative contributions made by beer, spirits and wines to the diet are disguised when each is recorded in terms of volume. Spirits have a very high energy content and 1 proof pint (equivalent to about 1.42 pints at 70° proof) provides about 7.5 MJ or 1,800 kcal, but essentially no other nutrient. Wines contain much less alcohol, even when fortified, and despite their

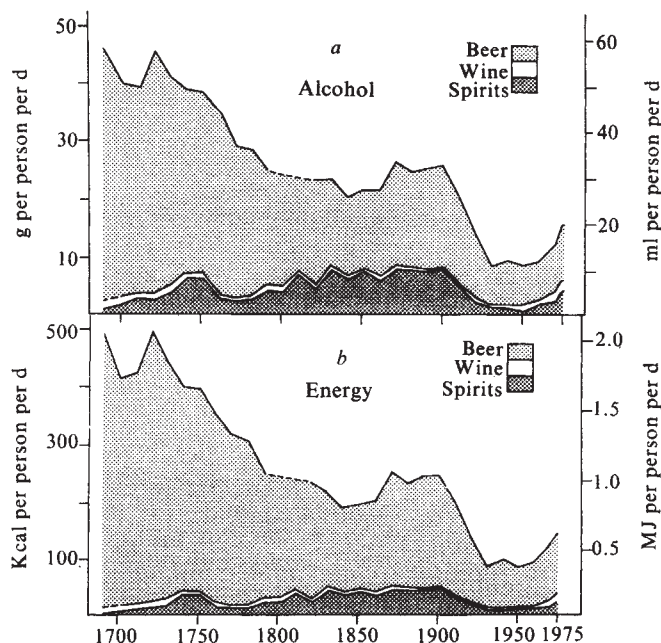


Fig. 2 Relative contributions of beer, spirits and wine to the diet; *a*, in terms of alcohol and *b*, in terms of energy. Values for 1821, 1831, 1951, 1961, 1971 and 1975 include the contributions made by cider and British wines. The alcohol and sugar contents of beer at original gravity 1.055° were each taken as 4.2 g per 100 ml (ref. 10); the alcohol content of spirits is fixed when quantities are recorded in proof gallons; and wines were calculated on the assumption that French, Rhenish and Empire or Commonwealth wines were 'light' and could be treated as an equal mixture of red, rose and white wines, while wines from Portugal, Spain and their colonies were 'heavy' and could be treated as an equal mixture of port, sherries and vermouth. The alcohol and energy values of these and cider were then derived from standard tables of food composition²⁰. Any errors in the assumptions necessary for wines are trivial while consumption has been so low.

sugar content they also provide less energy. The small amounts of iron and other nutrients are of little significance when the low consumption is averaged over the whole population. Beer contains the least alcohol, but in the amounts drunk its contribution to the energy (with 1 pint as drunk now providing between 0.6 and 1.7 MJ) and B-vitamin content of the diet can be substantial.

Figure 2 shows the amounts of alcohol and of energy provided by beer, wines and spirits. Although they are expressed per head of the population, each has been restricted to certain sections of the population, each of which would have drunk much more than the averages illustrated. Even so, it is obvious that the total amount of alcohol available has declined fairly steadily. Beer has always provided the most alcohol although the percentage has in general declined and now stands at 67%. Spirits have never provided more than a third of the total alcohol available; they now provide about 20%. Wine has recently become relatively more important.

The energy provided has changed almost exactly in parallel with the alcohol content (Fig. 2). The main difference is that spirits, which contain little or no carbohydrate, make a relatively smaller contribution to energy than to alcohol. The three beverages together provided between 1.5 and 2.5 MJ (400–500 kcal) per person per d in the first half of the eighteenth century, but this had fallen to less than 1 MJ (200 kcal) 100 yr later. After a rise to just over 1 MJ (250 kcal) in the latter part of the nineteenth century, it remained at an historical low of about 0.4 MJ (100 kcal) from 1930 to 1960 but has now increased again to about 0.6 MJ (150 kcal) per person per d.

Beer is and always has been the greatest source of energy as alcohol (on average) although its contribution has fallen from 92% of the total from all alcoholic drinks in 1731 to 77% in

1831, and from 84% in 1931 to 79% in 1971. Spirits have provided up to one-fifth of the total, while the contribution from wine, although small, has increased from 5% in 1731 (when most of the wine was fortified) to 9% in 1971 despite the relatively recent increase in the proportion of light wines with only two-thirds the energy value of their fortified counterparts.

It is not possible to calculate the relative importance of food and alcohol to the diet for most of the period covered, because few national surveys of the total food supply were attempted before 1940. We have, however, estimated the average energy requirement of the population at selected times, to determine what proportion of this was likely to have been satisfied by alcoholic drinks. The different age and sex structures of the population (particularly the greater proportion of children in earlier years) were taken into account from Census data, and present recommendations¹⁹ for each group were then applied to these on the assumption that in the nineteenth century all men and women were 'very active' and in the twentieth century they were 'moderately active'. The average requirement of the population was then approximately 10.3 MJ (2,460 kcal) per person per d in 1821, 10.5 MJ (2,500 kcal) in 1871 and 10.8 MJ (2,570 kcal) in 1911 compared with about 9.8 MJ (2,350 kcal) in 1971; the proportions met by the alcoholic drinks available thus averaged 10% in 1821 and 1871 and 8% in 1911, compared with about 5% in 1971.

Table 4 shows the amounts of three minerals (iron, calcium and magnesium) and two B vitamins (riboflavin and nicotinic acid) estimated to have been provided by the beer available in selected earlier years. The minor contributions of the different wines to iron intakes are also included, but the amounts of other nutrients provided by alcoholic drinks were insignificant.

Values for 1971 were derived from standard tables of food composition²⁰. Earlier values are the best estimates we can make in the virtual absence of direct information, and were obtained as follows. The B vitamins in beer, being derived mainly from the malt, seem to be related to the strength of the brew; thus, a sample of strong ale supplied to Queen's College, Oxford before World War II contained 0.39 mg riboflavin per 100 ml (ref. 2) compared with 0.06 mg in strong ale and 0.04 mg in draught bitter brewed recently²⁰. Other analyses of beer in the 1930s and 1940s showed more riboflavin and nicotinic acid than in modern beers—0.08 mg per 100 ml (including one bottle brewed in 1798)²¹ and 1.5 mg per 100 ml (ref. 22) respectively. These values were used in assessing intakes for 1941 and earlier. The vitamin and mineral contents of beers in previous centuries have thus been related to their original gravity.

Given these assumptions beer (and wine) seems to have made a major contribution to the nutritional value of diets in the seventeenth and eighteenth centuries, and especially to riboflavin, nicotinic acid, calcium and magnesium intakes.

We thank the librarians of the Customs and Excise Department for their help in making so many old records available, and the Trade Associations and many brewers and other individuals who provided information and advice.

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Interparticle forces in multiphase colloid systems: the resurrection of coagulated *sauce béarnaise*

C. M. Perram*, C. Nicolau† & J. W. Perram‡

Châlet Friedheim, 6386 Wolfenschiessen, Nidwalden, Switzerland

The successful preparation of sauce béarnaise is reported. The physico-chemical factors influencing the stability of this colloidal system are considered.

VARIOUS authors have extensively described^{1–5} the preparation of *sauce béarnaise* (SB), the colloquial name for a (hopefully) stable colloidal suspension consisting of a hydrophobic phase suspended in a low pH aqueous phase at different ionic strengths. It is highly probable that the colloidal particles are micelles consisting of a mixture of phospholipids, fats, proteins, cholesterol and various long-chain unsaturated fatty acids. The aqueous phase contains mainly acetic acid and sodium chloride at ionic strengths determined by the initial conditions. Chlorophyll-containing additives, we believe, have little influence on the colloidal properties of the system.

We shall first give a brief description of the underlying physico-chemical principles. Hydrophobic colloidal suspensions exist because of an interplay of double-layer repulsion and attractive dispersion forces. The latter are relatively independent of the composition of the dispersive medium and temperature although in some circumstances this assumption cannot be justified^{6–9}. The double-layer repulsions arise from the surface charge resulting from ionisation of adsorbed acetic acid, in this case, screened by the dispersing electrolyte solution¹⁰. This contribution to the forces can clearly be influenced by the conditions prevailing in the dispersive medium. The aim of this article is to indicate how an understanding of this interplay of forces can lead to more complete experimental control of the stability of this extremely complex and important system.

Theory

Although we can find no definitive light or neutron scattering data on this system, we assume that the micelles are spherical, and hence that the Van der Waals forces between them are well described by the equation of Hamaker¹¹

$$U_A = -\frac{A}{6} \left[\frac{2}{a^2 - 4} + \frac{2}{a^2} + \ln \frac{a^2 - 4}{a^2} \right] \quad (1)$$

where U is the interaction energy relative to infinite separation, a is the non-dimensional interparticle separation scaled with respect to the particle radius and A is the so-called Hamaker constant, with a value of about 10^{-12} erg. The number of adsorbed acetic acid molecules per unit area of the particles may be predicted from the ambient bulk concentration and a suitable adsorption isotherm^{12,13}. The fraction of these which are dissociated will naturally depend on the composition of the dispersive medium,

particularly the pH. Furthermore, we can expect that the solvation by acetic acid of the ionic groups on the colloid particles will significantly affect the stability of the particles¹⁴. If there are N_s dissociated acid groups per unit area these contribute a surface charge

$$\sigma_N = N_s q \quad (2)$$

where q is the protonic charge. The ionic strength

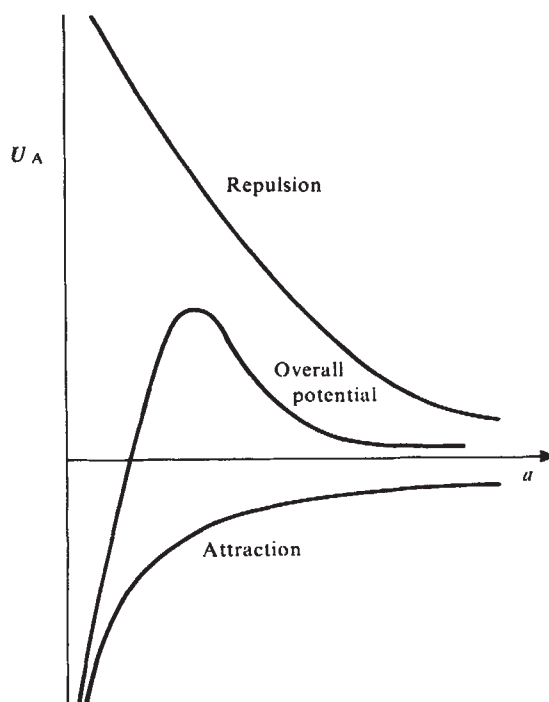
$$\mu = \frac{1}{2} \sum_{i=1}^N \rho_i z_i^2 \quad (3)$$

of the dispersing medium will result in a screening of the coulombic repulsion due to the negative charges on the particles' surfaces. The potential of these forces decays with distance r according to an exponential law¹⁵:

$$F \sim \psi_0^2 [1 - \tan h \kappa L/2]$$

where: ψ_0 = surface potential, κ = inverse Debye length, and

Fig. 1 Potential curves for the interparticle interaction in a multiphase colloid system.



*Permanent address: Julagervænget 1, 5260 Odense, Denmark.

†Permanent address: Institut für Strahlenchemie, Max-Planck Institut für Kohlenforschung, Mülheim/Ruhr, FRG.

‡Permanent address: Department of Mathematics, University of Odense, 5260 Odense, Denmark.

Table 1 Consistency of *sauce béarnaise*

Observer no.	No. of samples	Completely homogeneous	Partially homogeneous	Heterogeneous
1	2	2	—	—
2	2	2	—	—
3	2	2	—	—
4	3	3	—	—
5	2	2	—	—
6	2	2	—	—
7*	2	2	—	—
8*	3	3	—	—
9*	3	3	—	—

*The authors.

 L = distance between the particle surfaces.

The various contributions and overall potential energy curve are shown in Fig. 1. If the height of the maximum in the potential curve is large compared to kT , where k is Boltzmann's constant and T the absolute temperature, then the sauce (SB) will be stable.

This maximum will be increased by the surface charge and reduced by increasing either temperature or ionic strength. This accounts for the empirical observation that the application of multivalent ions has never been recommended in the manufacture of *sauce béarnaise*. Only the increase of σ can have a beneficial influence on the stability of the product.

Experimental methods

Commercially available reagents (vinegar, onion, egg yolks, butter, parsley, tarragon, *herbes de Provence*, mustard, pepper, NaCl and alpine water (H_2O)) were used, according to ref. 3. We do not expect that our conclusions would have been altered if the alternative procedures^{1,2,4,5} had been used. In our hands, heavy

coagulation was observed despite vigorous stirring and careful temperature control (± 5 K). When coagulation occurs, other authors recommended discontinuation of the experiment¹⁻⁵. However, based on the above theoretical considerations, we decided to add, with extremely vigorous stirring, a further quantity of the commercially available acetic acid solution, and the results confirmed our theoretical predictions.

Results and discussion

After addition of acetic acid, and as vigorous stirring proceeded, the heterogeneous phase soon assumed the expected homogeneous consistency. Examination of the resulting preparation was immediately undertaken by a significant number of trained observers. The results of this examination are listed in Table 1. To our knowledge, this is the first successful attempt to resurrect a *sauce béarnaise* based on the theory of the stability of lyophobic colloid¹⁰.

Thanks are due to Drs K. Hildenbrand, A. Reimann and to M. Hildenbrand, V. Hallmann, H. Dittrich and I. Hengst for agreeing to take part in the experiment.

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articles

New data on climatic trends

G. J. Kukla

Lamont-Doherty Geological Observatory, Columbia University, Palisades, New York 10964

J. K. Angell & J. Korshover

Air Resources Labs., ERL, NOAA, Silver Spring, Maryland 20910

H. Dronia

Wetteramt Hannover, D-3000, Hannover 42, FRG

Indicators of large-scale climate developments show that the oscillatory cooling observed in the past 30 yr in the Northern Hemisphere has not yet reversed. This conclusion was reached by updating our data on the month-to-month, season-to-season, and year-to-year variations of selected zonally averaged meteorological parameters.

THE reported set of climatic indices shown in Figs 1 and 2 represent large segments of the Earth-atmosphere system in both hemispheres. Changes in the average surface air temperatures in

M. Hoshiai

Laboratory of Physics, Aichigakuin University, Nisshin-cho, Aichi-gun, Aichi-ken, Japan

J. Namias

Scripps Institution of Oceanography, La Jolla, California 92037

M. Rodewald

D-2000 Hamburg 70, Rantzaustrasse 78, FRG

R. Yamamoto & T. Iwashima

Geophysical Institute, Kyoto University, 606 Kyoto, Japan

the Northern Hemisphere from 1951 to 1975 are shown in Figs 3-5 by curves 101-111, prepared by R. Yamamoto *et al.*^{1,2}. Curves 101-105 show the relative departures of the 12 month running means for designated latitudinal zones. Curves 106 through 111 in Figs 4 and 5 show the departures for spring (March-May), and autumn (September-November) respectively. Data from 358 stations, north of 20°S (see Fig. 1) were analysed. They were obtained from the *World Weather Records*³ and *Monthly Climatic Data for the World*⁴ and calculated as departures from the 1951-75 mean. An interpolating method of cubic spline under tension, developed by Cline⁵, was used in the

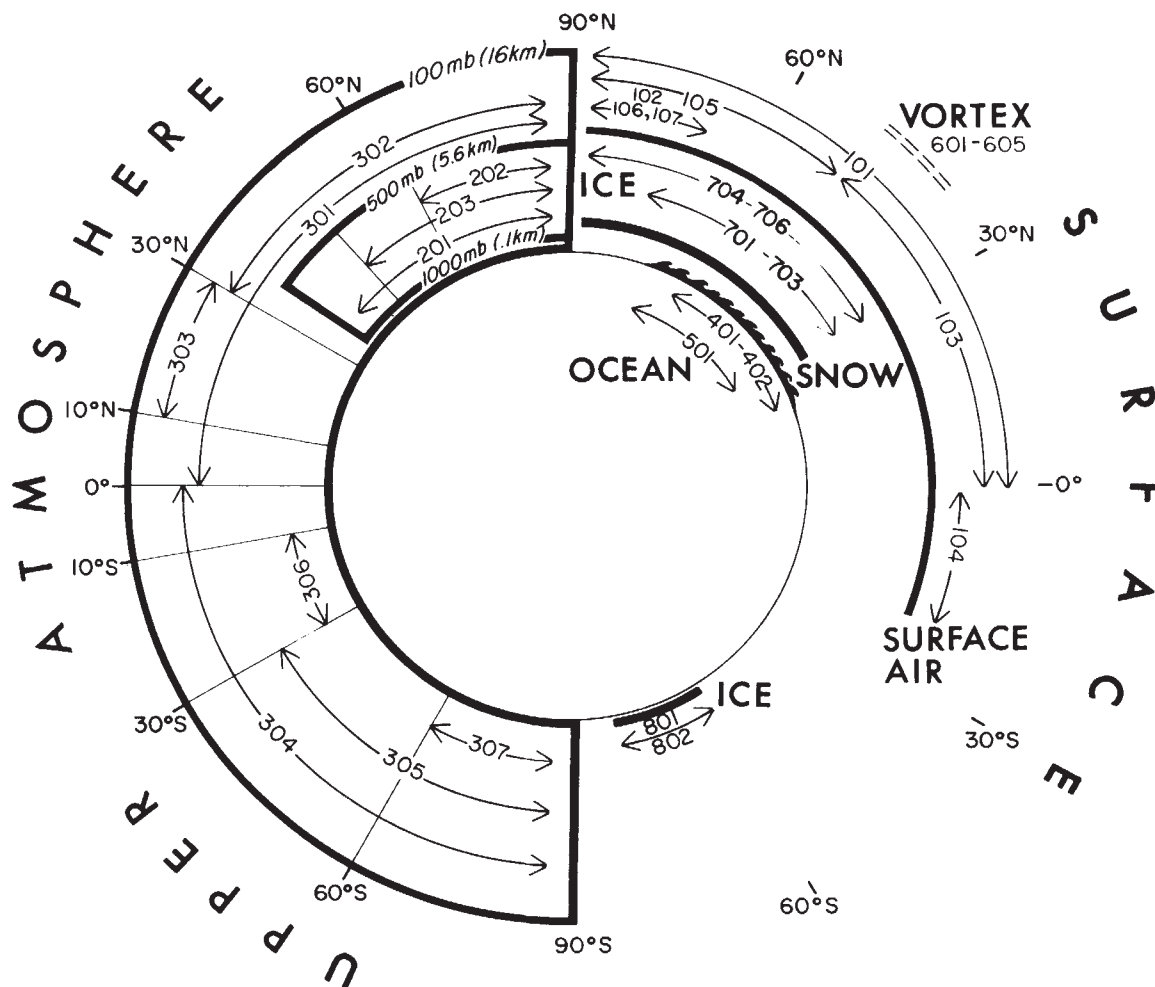


Fig. 1 Area represented by climatic indices listed in Table 1

analysis. The details of the averaging technique are given elsewhere^{1,2,5}. Frequently climate is defined by parameters measured 1.5–2 m above the ground, where most of the meteorological instruments are situated. Because the surface air temperatures correspond directly to this level, curves 101–111 are the most direct indicators of surface climate fluctuations included in our set. But, the large geographic variability in surface temperature trends and uneven distribution of observing stations severely limits the accuracy of our estimates. The results should be most reliable for the middle and high latitudes of the Northern Hemisphere where the station density is highest. The striking feature of the record is the large range of year-to-year temperature fluctuations. A progressive temperature drop over the recorded interval with oscillations about 3–4 yr long and an intensive cooling episode between 1961 and 1964, can be seen. The data are in general agreement with earlier work^{6–11,46–49}. It is interesting that during the past decade the high latitudes of the Northern Hemisphere show warming in the spring (Fig. 4, curve 106) but cooling in autumn (Fig. 5, curve 107). Only a slight change in the spring and autumn temperature is seen south of 50°N.

Free atmosphere below 500 mbar

The relative temperature of the free atmosphere between the 500-mbar and 1,000-mbar levels in the middle and high latitudes of the Northern Hemisphere from 1949 to 1976 was studied. The annual average departures from the 1949–73 mean are plotted in curves 201–203 in Fig. 3. The data were obtained by H. Dronia^{12,13} through the analysis of monthly pressure charts¹⁴. Approximately the lower 5.5 km of the troposphere is described. Information on the heights of the 500-mbar and 1,000-mbar levels was read at 220 gridpoints at intervals of 5° latitude and 10° longitude,

which cover about 55% of the hemisphere (Fig. 2). The average relative temperature was calculated from the changing thickness of the atmospheric column between the two limiting pressure levels. A decrease in thickness of 20 m corresponds to an average temperature drop of 1 °C. A detailed discussion of the method is given elsewhere^{12,13}. The data are more spatially homogeneous than ground level temperatures. It is possible that the upper atmosphere data include a small, as yet unknown 'instrumental' contribution to the cooling trend caused by gradually improved USSR radiosondes. This would lessen the average rate of the cooling trend as observed since the 1950s. Further studies seem necessary³⁴.

Perhaps the most striking feature of the record is the large year-to-year variability. The curves show a gradual oscillatory cooling through the studied interval. A record negative departure in the mid-latitudes (Fig. 3, curve 201) accompanied by relative warming in high latitudes (curve 202) occurred in 1976. Rapid cooling of the atmosphere between 1959 and 1964, first reported by Starr and Oort¹⁵ is also evident.

Free atmosphere below 100 mbar

The relative temperature of the free atmosphere between the 100-mbar level and surface from 1958 to 1976 was analysed by Angell and Korshover^{16,17}. The data are shown separately for different latitudinal belts of both hemispheres by curves 301–307. The Southern Hemisphere south of 30°S is represented by only 12 stations so that the results are highly tentative. The fluctuations in atmospheric temperatures north of 30°N are probably more accurately indicated by Dronia's curves 201–203, despite the fact that these curves refer only to the column below 500 mbar. But

Table 1 List of plotted indices

Curve	Parameter	Area	Units	~ Height	Interval	Average	Authors	Figure
101	SAT*	0–90°N	0.2 °C	—	1951–75	12-month run	Yamamoto Iwoshima Hoshiai	3a
102	"	70–90°N	"	—	"	"		3a,b
103	"	0–50°N	"	—	"	"		3a
104	"	0–20°S	"	—	"	"		3a
105	"	50–90°N	"	—	1965–75	"		3b
106	"	70–90°N	"	—	"	Spring		4
107	"	70–90°N	"	—	"	Fall		5
108	"	50–90°N	"	—	"	Spring		4
109	"	50–90°N	"	—	"	Fall		5
110	"	0–50°N	"	—	"	Spring		4
111	"	0–50°N	"	—	"	Fall		5
201	ATM†	35–90°N	0.2 °C	1,000–500 mbar (0.1–5.6 km)	1949–76	Annual	Dronia	3a
202	"	65–90°N	"	"	"	"		3a,b
203	"	50–90°N	"	"	1965–76	"		3b
301	ATM	0–90°N	0.2 °C	Surface–100 mbar (0–16.0 km)	1958–76	Annual	Angell Korshover	3a
302	"	30–90°N	"	"	"	"		3a
303	"	10–30°N	"	"	"	"		3a
304	"	0–90°S	"	"	1958–75	"		3a
305	"	30–90°S	"	"	"	"		3a
306	"	10–30°S	"	"	"	"		3a
307	"	60–90°S	"	"	"	"		3a
401	SST‡	N. Central Pacific	0.2 °C	—	1947–77	12-month run	Namias	3a
402	"	"	"	"	"	Monthly		6
501	SST	N. Atlantic Stations (NOAS)	0.2 °C	—	1951–72	5-yr run	Rodewald	3a
502	"	Central N. Atlantic (Station C)	"	—	1966–76	"		3a
503	"	"	"	—	1967–76	12-month run		3b
504	"	Boothbay Harbor	"	—	1965–76	Annual		3b
601	VOR§	N. Hem.	Percent	300 mbar (9.2 km)	1966–76	12-month run		3b
602	"	"	"	"	"	Spring	Angell Korshover	4
603	"	"	"	"	"	Fall		5
604	"	"	"	"	"	Summer		4
605	"	"	"	"	"	Winter		5
701	SNO¶	N. Hem.	Millions km ²	—	1966–77	12-month run	Matson Wiesnet	3b
702	"	N. America	"	—	"	"		3b
703	"	Europe Asia	"	—	"	"		3b
704	SIC	N. Hem.	Millions km ²	—	1967–77	12-month run	Kukla	3b
705	"	"	"	—	1967–76	Spring		4
706	"	"	"	—	"	Fall		5
801	ICE**	S. Hem.	Millions km ²	—	1973–77	12-month run		3a
802	"	"	"	—	"	End of Nov.		3a

Temperature departures:

*SAT, Surface air.

†ATM, upper atmosphere.

‡SST, Sea surface.

Area changes:

§VOR, relative vortex area (%).

¶SNO, Snow on land.

||SIC, Snow and pack ice.

**ICE, Pack ice.

the latter are based on data from a larger number of stations. The average annual heights and temperatures of the analysed pressure levels were computed from the *Monthly Climatic Data for the World*⁴. The changes of average thickness were converted to mean annual temperature departures based on assumptions similar to those made by Dronia¹². Details of the procedure are described elsewhere¹⁰.

Apart from the large year-to-year changes, a gradual oscillatory drop of atmospheric temperatures is observed in all curves except the 60–90°S, which shows warming. Data for zones poleward of 30°N are in general agreement with Dronia's curves 201–203 (Fig. 3). Large oscillations but no pronounced trend can be seen in the low latitudes of both hemispheres between 30°N and 30°S. Angell and Korshover¹⁷ reported essentially no change in the temperature of the global atmospheric column (90°N–90°S) averaged with respect to mass between 1971 and 1976.

Pacific sea-surface temperatures

Sea-surface temperatures (SST) in the North Central Pacific are shown for 1947–77. The data were analysed by Namias¹⁸ as

departures from the 1947–66 mean. They are shown in curves 401 (Fig. 3) and 402 (Fig. 6). The time series is based on ship observations received by the National Marine Fisheries Service of NOAA. Over 20,000 ship injection temperatures for each month were averaged separately for 2° × 2° Marsden squares, analysed, and then smoothed on to a 5° × 5° grid centred at the intersection point. Data for the resulting 153 oceanic grid points were weighted by the relative area at each latitude and averaged. Details of the data collection and averaging are described by Namias^{18,19}.

The large month-to-month and year-to-year variability is the outstanding feature of the record. The progressive oscillatory drop of average temperatures from approximately 1959, the increased amplitude of fluctuations from 1963 onwards (Fig. 6), pronounced cooling between 1963 and 1964, and 1968 to 1972, marked warming between 1965 and 1967, and the extreme departure of August 1976 temperatures from normal are also apparent. The largest negative departures frequently occurred in the warmest season between July and October (Fig. 6).

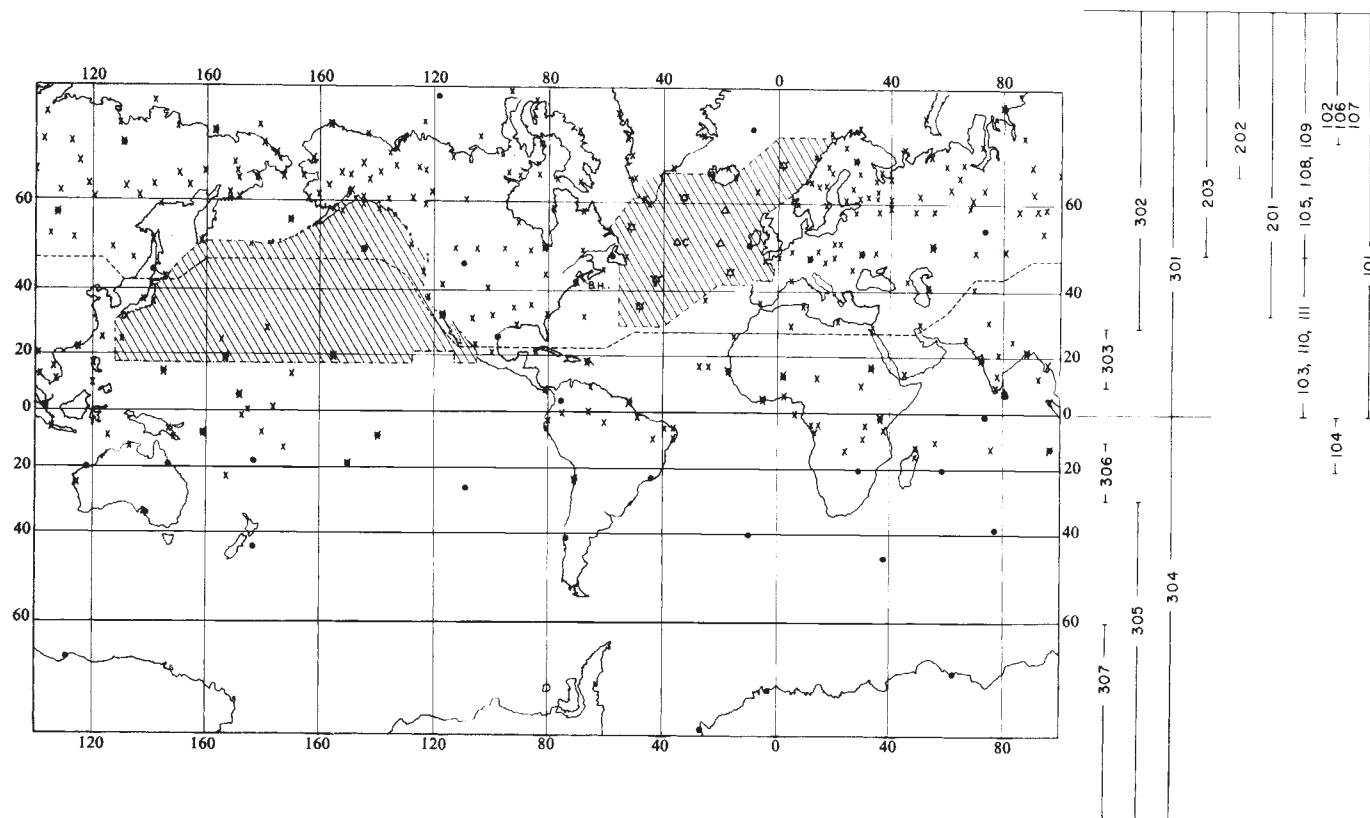


Fig. 2 Station network (polewards of 75° not shown). ×, Air surface temperature (series 100); ●, upper atmosphere temperatures (series 300); △, ship stations; BH, coastal station Boothbay Harbour. Hatched areas, sea surface represented by curves 401, 402, and 501; dashed line, southern border of the area represented by curve 201.

The secular change in sea-surface temperature is not evenly distributed⁴¹. During the winter of 1977, for example, a cold anomaly of -2°C was centred around 180°W and 40°N , and also affected coastal waters off Alaska and continental United States. At the same time in the vicinity of 130°W and 40°N the water was warmer than the long-term mean by 1.5°C . The records available from United States coastal stations²⁰ show a relatively warm ocean in 1957 and 1958 but generally cold from 1971 onwards. The widespread cold anomalies of coastal SST occurred in 1933–34 and 1943–44 but these were not as intense and long lasting as in the 1970s.

Atlantic sea-surface temperatures

Sea-surface temperatures in the North Atlantic from 1951–1976 were analysed by Rodewald²¹ who averaged the information collected from nine North Atlantic Stations (NOAS) (Fig. 1). The results are shown by curve 501, in Fig. 3a as 5-yr running means. After withdrawal of US weather ships in 1973, only two of the original nine observing stations remained occupied, C and M. Comparison of station C's record before 1973 with that of the NOAS stations shows a parallel slope in the pentadal running means (curve 502, Fig. 3a). Station C is, therefore, considered an example, to some extent, of the general trend in the North Atlantic after 1973 (curve 503, Fig. 3b). Curve 504 from Boothbay Harbor, Maine, represents the changes observed along the northeastern seaboard of North America²². Curves 501 and 502 combined, indicate a progressive drop in average SSTs, which from 1951 through 1976 amounted to approximately 0.75°C . The drop was interrupted by a temporary warming between 1963 and 1966. The largest cold departures for station C (curve 503) were observed in summer, between June and September.

Polar vortex extent

The area of the 300-mbar northern circumpolar vortex between 1966 and 1976 was measured by Angell and Korshover^{23,24}. The area north of the main belt of westerlies at 40° – 50°N latitudes was

identified and planimeted. The contours were taken as 9,280 m for spring and autumn, 9,120 m for winter, and 9,440 m for summer, as determined from the mean monthly polar stereographic maps issued by the Free University of Berlin.

Figures 4 and 5 show that in the spring, summer, autumn and winter of 1976 the vortex area was the largest for the 10 years of record. The vortex area for the 1976–77 winter was 4% larger than the average for previous winters and more than 1% larger than any previous winter observed. The total area for 1976 was well above average and was significantly larger than the previous peak in 1968.

Quiroz²⁵ studied the central height of the polar vortex at the 10-mbar level for the winter seasons of 1966–76. He found the values to be relatively high (close to 29 km) in the winters of 1968–69, 1969–70, and 1972–73, but low (close to 28 km) in 1966–67, 1971–72 and particularly low in 1975–76. Data for the winter of 1976–77 have not been reported yet.

Northern Hemisphere snow and ice

The seasonal and year-to-year variation of the area covered by snow in the northern hemisphere is shown in Figs 3b–5, by curves 701–706 with increasing area plotted downwards. The data are an update of measurements published earlier^{26–30}. The area is expressed in millions of km^2 and includes snow and pack ice of three classes of relative reflectivity as delimited in the NOAA weekly charts³⁰. The maps are prepared from a visual interpretation of 6 d of satellite imagery in visible light and infrared. Details are given in refs 26–29. Interpretation of satellite imagery is not without problems⁵⁰.

The large year-to-year variability, considerably more pronounced in Eurasia (curve 703) than in North America (curve 702), is the striking feature of the record. There were only minor changes in the North American winter extents. The greatest variance was observed in autumn (Fig. 5) and in Asia (Fig. 3b). It can be also seen that the autumn extent of snow and ice gradually increased between 1967 and 1972 and decreased between 1973 and

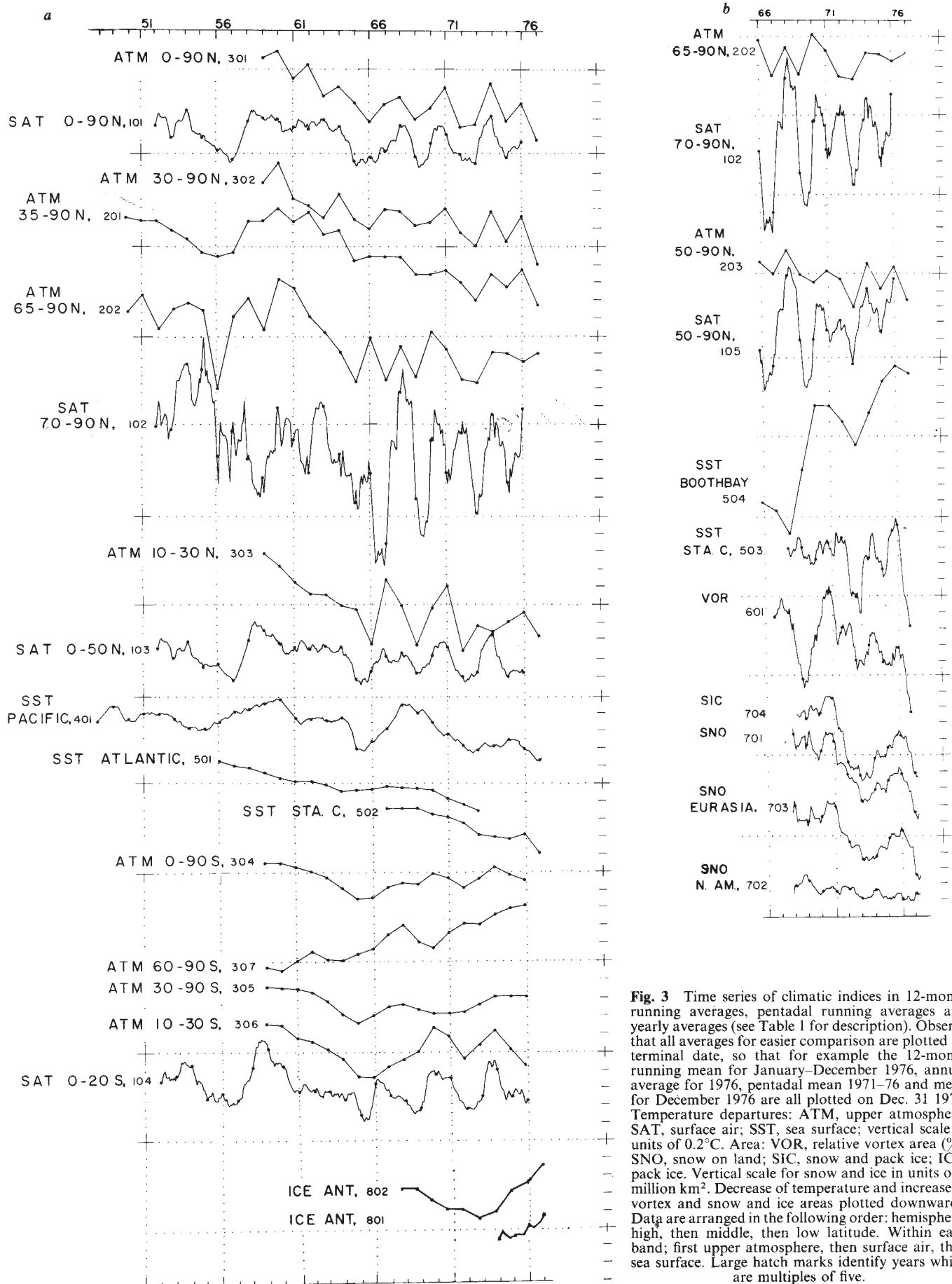


Fig. 3 Time series of climatic indices in 12-month running averages, pentadal running averages and yearly averages (see Table 1 for description). Observe that all averages for easier comparison are plotted on terminal date, so that for example the 12-month running mean for January–December 1976, annual average for 1976, pentadal mean 1971–76 and mean for December 1976 are all plotted on Dec. 31 1976. Temperature departures: ATM, upper atmosphere; SAT, surface air; SST, sea surface; vertical scale in units of 0.2°C . Area: VOR, relative vortex area ($\%$); SNO, snow on land; SIC, snow and pack ice; ICE, pack ice. Vertical scale for snow and ice in units of 1 million km^2 . Decrease of temperature and increase of vortex and snow and ice areas plotted downwards. Data are arranged in the following order: hemisphere; high, then middle, then low latitude. Within each band; first upper atmosphere, then surface air, then sea surface. Large hatch marks identify years which are multiples of five.

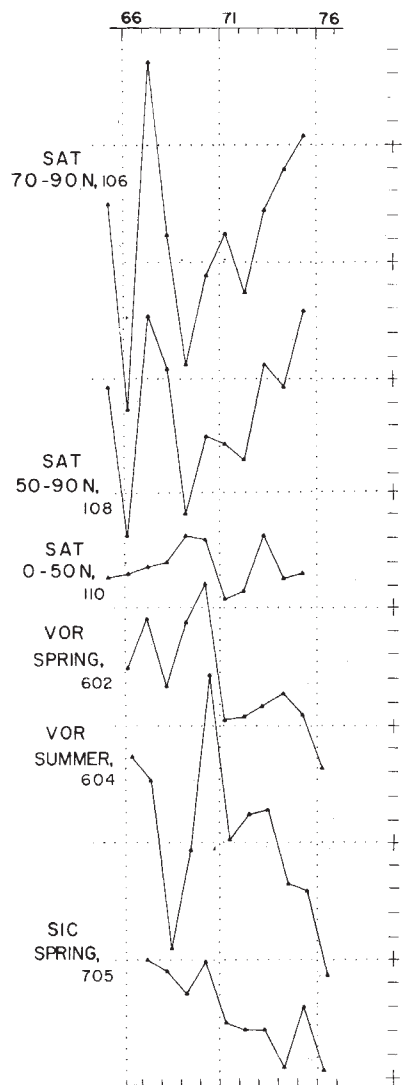


Fig. 4 Average climatic indices for spring (March–May) and vortex area for summer (June–August). Symbols and scale as in Fig. 3.

1975 (Fig. 5). The spring totals, however, increased throughout most of the interval from 1966 to 1975. Spring snow has a large impact on the surface heat balance of the continents because it reflects a significant part of incoming radiation which at this time of the year is high. Thus, the increased spring snow cover of the few past years has meant, in general, a decrease in the amount of solar radiation absorbed by the Earth's surface.

While our satellite-derived data show snow and pack-ice area combined, Sanderson³¹ measured the sea-ice extent alone in different sections of the Arctic between 1966 and 1974 using the *Monthly British Ice Charts*³². The data indicate a decrease in the winter and spring pack-ice area in most sectors after 1969. This decrease is especially pronounced in February and April. Haupt and Kant³³ made similar observations on pack ice in the Barents Sea. There the maximum spring coverage occurred in 1960 and the minimum in 1973. Thus the heavy snow season of 1972–73 was associated with a relatively open Arctic Ocean and in particular with a relatively ice-free Barents Sea.

Antarctic pack ice

The pack-ice area is shown by curves 801 and 802, in Fig. 3a (increasing area downwards). The data were analysed by Kukla²⁷. Information on sea ice in the Antarctic waters was obtained from charts routinely produced in weekly intervals by the Fleet Weather Facility (FLEWEAFAC) of the US Navy, based on interpretation of satellite images obtained from NOAA,

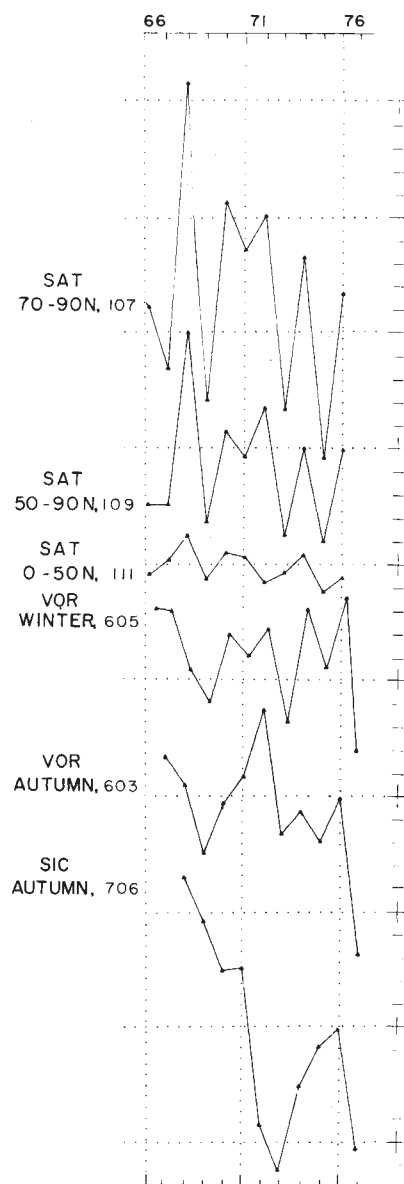
NASA, and DMSP polar orbiting satellites. Both curves show the total area of ice with a concentration greater than 1 octa.

Antarctic pack ice extent during selected seasons of the 1966–72 period was charted and studied by Stretten³⁶, Sissala *et al.*³⁷, and Ackley and Keliher³⁸. We reconstructed and measured additional charts using visible and infrared images from NOAA satellites. We also measured 1971–72 summer charts prepared by the US Navy. Monthly ice extents for the 1967–76 interval are greater than those reported by Treshnikov³⁹ for the late 1950s.

Comparison of curves 801 and 802 shows that the change of the mean extent of pack ice in November from 1972–76 roughly parallels the change in the average annual cover. Curve 802 for the end of November is, therefore, considered indicative of the average annual secular variations of pack ice cover between 1966 and 1972.

The November extent of pack ice increased between 1966 and 1972 but decreased from 1974 to 1976. Between 1967 and 1975 the Southern Hemisphere pack-ice variation (Fig. 3a, curves 801, 802) parallels the variation in Northern Hemisphere snow and ice cover (Fig. 3b, curve 704), as does the decrease in 1974 and 1975. The 1976–77 Antarctic ice season, however, continued to be light, while the snow coverage in the Northern Hemisphere was heavy. Thus, except for 1976–77, fluctuations in the surface extent of the

Fig. 5 Average climatic indices for autumn (September–November) and vortex area for winter (December–February). Symbols and scale as in Fig. 3.



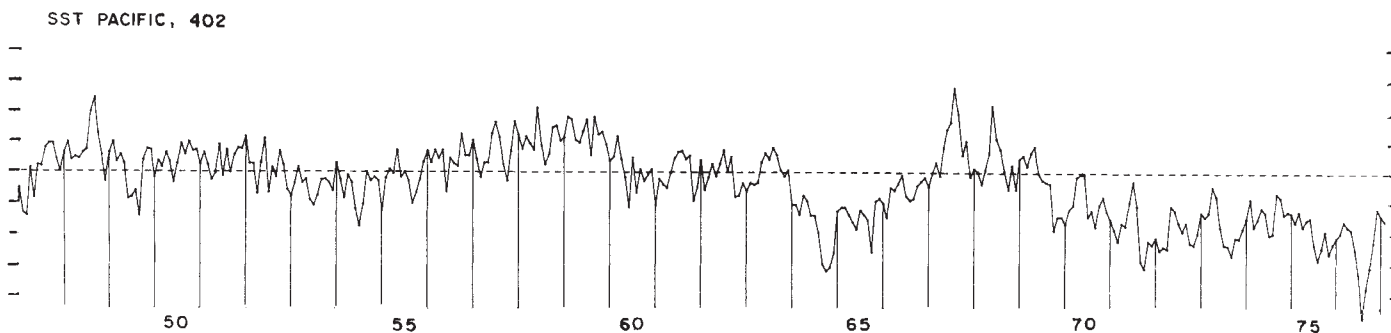


Fig. 6 Monthly departures of average sea surface temperatures in the north central Pacific from the 1947-66 mean. Vertical Scale in units of 0.2°C .

cryosphere in the two hemispheres for the 1967-76 period, seem to be generally in phase.

Discussion

The quality of individual records used in our study varies depending on the density of the station network, and frequency of observations. Also some of the data, for example, the upper atmosphere temperature and pressure readings, may have been influenced by gradual improvements of instrumentation. In general, observations made on the continents of both hemispheres are the most reliable. Temperature changes of the ocean surface, although of extreme importance in the climate system, are reliably known only over a fraction of the world's oceans, namely the north central Pacific, the central part of the North Atlantic and indirectly in the subpolar seas. Vast areas in the mid-latitudes of the Southern Hemisphere have remained virtually unobserved. Hence, conclusions on climate changes in the Southern Hemisphere and for the globe are much less reliable than those for the Northern Hemisphere. Also, our averaging techniques do not allow for descriptions of local trends in weather parameters which may be vastly different from mean zonal changes, and which are the most readily observable features by the population of affected regions⁵¹. Nevertheless, by comparing records of several independent climatic indices from different geographic areas and observing the fluctuations on a month to month basis, we were able to provide a cross check of most conclusions.

Summarising the data presented in Figs 3-5, the following observations can be made. (1) The year-to-year temperature fluctuations which compose most of the observed variance in our zonally average data, are in phase over most of the Northern

Hemisphere and also over parts of the Southern Hemisphere.

(2) The range of the year-to-year variability in most data sets is several times larger than the departure due to the long-term trends^{10,11,51}. In this situation, reliable identification of trends or recognition of a trend reversal requires parallel observations made on different elements of the climate system over sufficiently long intervals⁵².

(3) The middle and low latitudes of the Northern Hemisphere show progressive oscillatory cooling throughout the recorded interval (Fig. 3a, Table 2). The high latitudes cooled until about 1965 and then show little change or warming.

(4) Pronounced cold episodes occurred globally in 1954-56; 1964-65, 1968, 1971-72, 1974 and 1976. The years 1958, 1959, 1970, 1973 and 1975 were comparatively warm. Several minima in curves 101 and 103 (Fig. 3) in the past 10 years reached the level of the single extreme recorded in the 1950s. Sea surface temperature minima were considerably lower in the 1970s than in the 1950s (Figs 3b, 6).

(5) From 1950 to 1975, the rate of cooling of most of the climatic indices in the Northern Hemisphere was between 0.1 and 0.2°C per decade (Table 2). The North Atlantic sea surface and the free atmosphere of the northern middle and high latitudes show the largest drop in temperature. The drop in the surface air temperatures was relatively small and given the large magnitude of the fluctuations over this period, the trend may not even be statistically significant.

(6) The slope of the five-year running means of most indices from the middle and low latitudes of the Northern Hemisphere increased between 1971 and 1975 with respect to the 1966-70 pentad. During the same interval in the high latitudes the slope

Table 2 Average slope of overlapping pentadal means in the 1951-75 interval

Northern hemisphere			Average slope in $^{\circ}\text{C}$ per decade				
Curve	Parameter	Area	1955-60	1960-65	1965-70	1970-75	Average
301	ATM	0-90°N	NO	NO	-0.068	-0.324	-0.196
101	SAT	0-90°N	+0.088	-0.204	-0.068	-0.088	-0.068
202	ATM	65-90°N	+0.556	-0.944	-0.300	-0.208	-0.224
102	SAT	70-90°N	-0.760	-0.428	-0.184	+0.412	-0.240
201	ATM	35-90°N	+0.328	-0.316	-0.620	-0.160	-0.192
203	ATM	50-90°N	+0.476	-0.476	-0.584	-0.264	-0.212
105	SAT	50-90°N	-0.156	-0.072	-0.368	+0.324	-0.018
401	SST	N. Pac.	+0.304	-0.492	+0.188	-0.512	-0.128
501	SST	N. Atl.	-0.480	-0.200	-0.260	NO	-0.313
303	ATM	10-30°N	NO	NO	+0.144	-0.316	-0.086
103	SAT	0-50°N	+0.156	-0.244	+0.028	-0.220	-0.070
Southern hemisphere							
304	ATM	0-90°S	NO	NO	+0.116	+0.068	+0.092
307	ATM	60-90°S	NO	NO	+0.396	+0.468	+0.432
305	ATM	30-90°S	NO	NO	-0.016	+0.180	+0.082
306	ATM	10-30°S	NO	NO	+0.272	+0.104	+0.188
104	SAT	0-20°S	+0.168	-0.508	+0.072	-0.112	-0.095

NO, No data. Values are arithmetic averages of five departures between successive overlapping pairs of pentadal means. Observe that, for example, the value for 1955-60 was obtained from pentadal means for 1951-55, 1952-56, 1953-57, 1954-58, 1955-59 and 1956-60. Result was multiplied by 10 to show the decadal rates.

decreased or reversed (Table 2). In 1971–75 the sea surface in North Central Pacific and North Atlantic was significantly colder (Fig. 3a), snow cover and vortex area significantly larger (Fig. 3b), and average temperatures of the free atmosphere in the low and middle latitudes significantly lower than in the previous pentad (Fig. 3a and b). Record departures frequently occurred in 1976 and in early 1977. Our data do not show a reversal in the cooling of the Northern Hemisphere. The short-term recovery between 1973 and 1975 did not continue through 1976.

(7) The spring (March–May) surface-air temperatures during the last decade increased in the northern high latitudes but showed little change in the low and middle latitudes. At the same time, the spring vortex area and snow cover gradually expanded (Fig. 4). The autumn (September–November) surface-air temperatures in the last decade dropped significantly in the northern high latitudes, and, to a lesser degree in the low and middle latitudes. Autumn snow cover increased substantially while the autumn and winter vortex areas showed less change (Fig. 5).

(8) Reliable conclusions on temperature trends in the Southern Hemisphere are not yet possible since data are extremely sparse. Lack of information for the 30–60° latitude belt is a critical deficiency. In this belt, so poorly represented in our data set, satellite mapping has revealed extensive negative departure in the 1976 sea-surface temperatures with respect to the previous year⁴².

Our data from the Southern Hemisphere show cooling until the mid 1960s, then slight warming (Table 2). In high latitudes, south of 60°S, the upper air gradually warmed throughout the recorded interval. The pack-ice area expanded between 1967 and 1973, and decreased thereafter. According to Damon and Kunen⁴⁰, the surface air in the Southern Hemisphere warmed between 1943 and 1974. Yamamoto *et al.*² found the 0–90°S surface air 0.03 °C cooler in the 1968–72 interval than in the pentad ending in 1962. Angell and Korshover¹⁷ reported that the free atmosphere in the early 1970s was cooler than in the late 1950s. In all the mentioned studies, however, the reported temperature departures are very small, data variability very large and station density insufficient.

(9) There are parallels in the climate development of the two hemispheres. First, the atmosphere in the high latitudes warmed during the last decade; second, snow and pack ice expanded in 1972 and 1973 but decreased in 1974 and 1975; third, accelerated cooling took place in the early 1960s.

We have reported changes of selected climatic indices zonally averaged over large segments of the globe. Possible causes of the fluctuations have been discussed elsewhere^{1,16,43–45}. Our zonally averaged indices have suppressed large spatial inhomogeneities which occur within any given latitude band. A coordinated investigation of these regional shifts of weather patterns, including the incidence of various weather extremes, will be our next objective.

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Conjugation proteins encoded by the F sex factor

Nicholas Kennedy*, Lothar Beutin & Mark Achtman

Max-Planck Institut für molekulare Genetik, Abt. Trautner, Berlin, FRG

Ron Skurray†

Department of Molecular Biology, University of California, Berkeley

Chimaeric plasmids carrying EcoRI fragments of the F sex factor have been used to identify proteins involved in conjugation and to assign them to tra cistrons. Most of these proteins are incorporated into the cell envelope and are individually regulated at the post-transcriptional level.

*Present address: Division of Virology, National Institute for Medical Research, Mill Hill, London NW7, UK.

†Present address: Department of Molecular Biology, University of Edinburgh, Edinburgh.

‡Present address: Institut für Medizinische Chemie der Universität Innsbruck, Austria.

Ursula Rahmsdorf‡ & Peter Herrlich

Max-Planck Institut für molekulare Genetik, Abt. Schuster, Berlin, FRG

THE transfer of genetic material between bacteria by conjugation is an important evolutionary mechanism by which bacteria adapt to a changing environment, and also provides a model system for analysing cell-cell interactions. Conjugation is mediated by a variety of sex factors: we have chosen to work with the F sex factor whose genetics are the best known. The genes required for the synthesis of F pili, stabilisation of the mating aggregates, DNA transfer, and surface exclusion are contained in a single large transcriptional unit¹. For simplicity this is called the 'tra operon'. In it some 13 cistrons have been genetically defined (see Fig. 1). Of these, nine (*traA*, *L*, *E*, *K*, *B*, *C*, *F*, *H* and part of *traG*) are

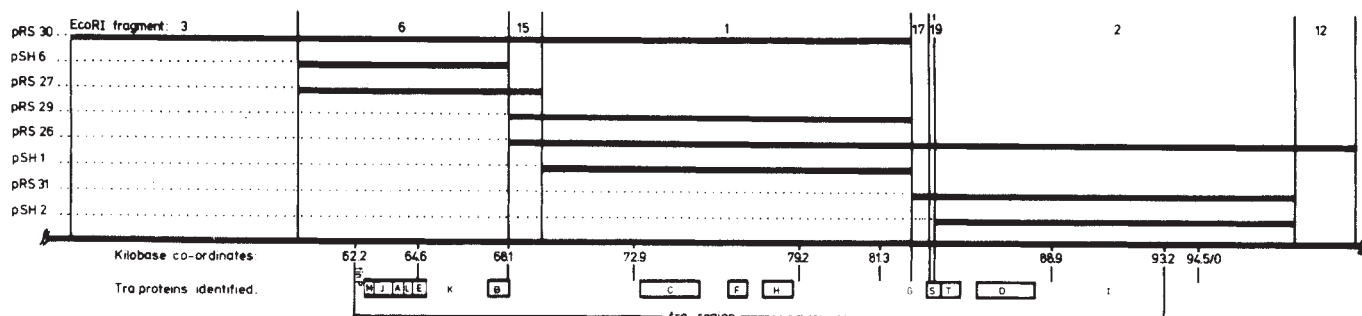


Fig. 1 Map of the F transfer region. Horizontal bars represent the fragments of F cloned in the corresponding chimaeric plasmids. The origin of DNA transfer is to the right of the 62.2 kb coordinate¹⁴. Proteins which we have identified are boxed. The lengths of the boxes correspond to the lengths of DNA required to code for the proteins. Except for *traM* and *traS*, about which there remains some ambiguity, the order and location of the cistrons shown have been demonstrated⁴. The pRS chimaeric plasmids were cloned⁹ using the plasmid vector pSC101 (ref. 23), and the pSH chimaeric plasmids were cloned using the plasmid vector ColE Amp (ref. 10a).

needed for the synthesis of F pili²⁻⁴. In addition, expression of the whole of *traG* is needed before F-carrying donor cells can form stable mating aggregates with F⁻ recipients⁵. An additional three cistrons, *traM* (which is outside the *tra* operon), *traD* and *I*, are required for DNA transfer^{2,4}. The *traS* and *T* cistrons convert F-carrying cells into poor recipients in conjugation with other F-carrying donors^{5,6} (the surface exclusion phenomenon). It seems that transcription of the *tra* operon is promoted by the product of the positive control gene *traJ* (refs 7,8). *traJ* is itself regulated by a repressor complex, the products of the *finP* and *finO* genes⁸. The *finO* gene is missing in the F sex factor, and so F *tra* genes are normally fully expressed.

All these cistrons have been genetically mapped relative to the endonuclease *EcoRI* sites of F (ref. 4) and chimaeric plasmids expressing the *tra* cistrons have been cloned^{4,9}. We have now used these chimaeric plasmids to make a comprehensive analysis of the protein products of the *tra* cistrons, as synthesised *in vitro* and in minicells. This identification of the conjugation proteins has also allowed us to examine their regulation and intracellular location.

Expression of *tra* cistrons by chimaeric plasmids

Two series of chimaeric plasmids have been constructed^{4,9} from *EcoRI* fragments of F DNA. The pRS series was constructed using the plasmid vector pSC101 (ref. 10) and the pSH series using ColE1Amp (RSF2124)^{10a}. Unlike F itself these plasmid DNAs segregate readily into minicells and can be purified in good yields. As shown in Fig. 1 the cloned DNAs of the pRS series each span one or more *EcoRI* sites in the *tra* region, whereas those of the pSH series do not. We were able to take advantage of this to detect possible hybrid peptides arising from read-through at the junctions between cloned and vector DNA. The chimaeric plasmids pRS27, pRS29 and pRS31 each carry approximately one third of the *tra* region, and each encodes relatively few *tra* proteins. These plasmids were used to identify the *tra* proteins as described below.

In our conditions, the only proteins synthesised in minicells were those directed by plasmid DNA which had segregated into the minicells. Minicells carrying chimaeric plasmids showed a linear increase in the incorporation of radioactively-labelled amino acids into proteins over the time period used. Analysis of these radioactive proteins by SDS polyacrylamide gel electrophoresis followed by autoradiography revealed the individual proteins encoded by the chimaeric plasmids, and the intensity of the bands corresponded to the net rates of synthesis.

Genetic complementation analyses⁴ have demonstrated that the inserted DNA of all these chimaeric plasmids is expressed in the cell. Since only chimaeric plasmids carrying *EcoRI* fragment 6 (f6) possess the *tra* operon promoter, which is located between *traJ* and *traA* (ref. 1), the others must depend on transcription initiated from promoters on the plasmid vectors for expression of the inserted DNA. In minicells the chimaeric plasmids directed the synthesis of specific protein bands which were characteristic of

the cloned *EcoRI* fragment. Figure 2 shows that under our conditions, few proteins were encoded by the parent plasmids pSC101 and ColE1Amp and that additional proteins were synthesised when chimaeric plasmids were used as templates. With the

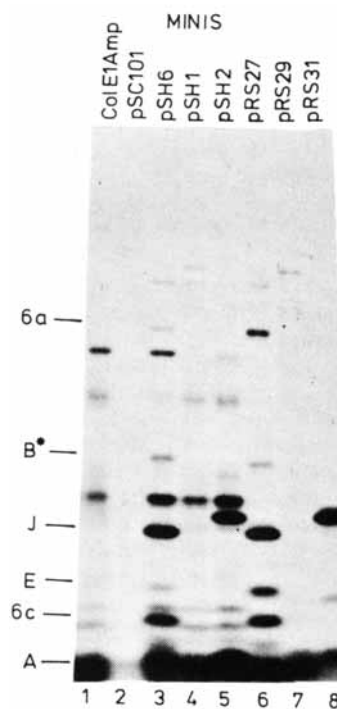


Fig. 2 Proteins synthesised in minicells from plasmid templates and analysed by SDS polyacrylamide gel electrophoresis. The plasmids present in the minicells are indicated above individual tracks on the autoradiogram. The positions of protein species subsequently identified (see Fig. 3) are marked at the sides of the figure. Note that TraAp (tracks 3 and 6) is overshadowed by low molecular weight material on this autoradiogram and that pSC101-encoded proteins are only visible after longer exposures. No protein synthesis was detected in minicells not carrying any plasmid DNA. All bacterial strains were derivatives of the Su⁻ minicell-producing strain DS410 (ref. 24). Minicells were purified by two successive sucrose gradient centrifugations²⁵ from 150 ml cultures grown to stationary phase in L broth²⁶. The preparations contained less than one viable cell per 10⁴ minicells. Minicell suspensions (6 × 10⁹ minicells in 1.2 ml of 56/2 minimal medium²⁶ supplemented with glucose and thiamine) were incubated for 60 min at 37 °C followed by a further period of 60 min in the presence of 25 μCi of U-¹⁴C protein hydrolysate (Amersham, 55 mCi mM⁻¹). The minicells were then pelleted by centrifugation, resuspended in 1 ml of L broth and incubated at 37 °C for a further 10 min. After washing with L broth, the minicells were resuspended in 100 μl of sample buffer²⁷, heated at 90 °C for 2 min and centrifuged for 2 min in an Eppendorf 3200 table centrifuge; the supernatant was analysed by SDS gel electrophoresis using 11 to 20% acrylamide gradients followed by autoradiography (fluorography) as described^{6,27}.

Table 1 The *tra* proteins

Cistron	No. of mutants affecting protein/No. of mutants tested*	Molecular weight of protein ($\times 10^{-3}$)†	Molar ratio‡		% bound to cell envelope¶
			<i>in vitro</i>	minicell	
<i>traM</i>	2/2	13	—	21	—
<i>traJ</i>	1/4	23.5	(100)	(100)	81
<i>traA</i>	3/3	13.7	141	26	96
<i>traL</i>	2/2	11	70	—	—
<i>traE</i>	2/2	19	22	17	88
<i>traK</i>	0/2	—	—	—	—
<i>traB</i>	2/2	29	7	12	67
<i>traC</i>	3/3	78	(20)	(20)	5–70
<i>traF</i>	3/3	25	49	17	75
<i>traH</i>	3/3	40	18	23	76
<i>traS</i>	3/3	18	7–170	22–58	76
<i>traT</i>	4/4	25	(112)	(112)	89
<i>traD</i>	2/2	77	2	3	5–70
<i>traI</i>	0/2	—	—	—	—

*In most cases when any one mutant was tested, a single protein band disappeared from both the *in vitro* and the minicell products (representative results are given in Fig. 3). The primary exceptions were that TraMp was only detected in minicells, traLp was only detected *in vitro* and that protein 6c was synthesised at a lower rate *in vitro* (only) using pRS27 DNA carrying *traJ90*. Three further exceptions have also been included as mutants affecting a protein: TraMp and TraTp were synthesised at lower than normal rates by mutants carrying the mutations *traM226* (on pRS27) and *traI249* (on pRS31), respectively; TraBp disappeared and was replaced by two protein bands, one slightly smaller and the other slightly larger, when the mutation *traB269* on pRS27 was analysed.

†Molecular weights were determined relative to the following protein standards, whose molecular weights $\times 10^{-3}$ are given in parentheses: RNA polymerase β' (165), RNA polymerase β (155), bovine serum albumin (68), catalase (60), aldolase (40), RNA polymerase α (39), bovine chymotrypsinogen (25.7), lysozyme (14.3), myoglobin (17.2), cytochrome *c* (11.7) and F pilin (10.7). All proteins were from Boehringer (Mannheim) except F pilin. The molecular weights of the unidentified proteins shown in Fig. 3 were: 6a (tracks 1, 5–8, 9–11) 55,000; 6b (tracks 1, 5, 9–11) 24,000; 6c (tracks 1–11) 16,000; 2a (tracks 21, 23–25) 25,500. All except 2a were cell envelope associated.

‡The intensity of individual bands on autoradiograms was evaluated by densitometry. For each chimera, these values were expressed relative to that determined for TraJp, TraCp or TraTp within the same track and after adjustment for the molecular weight. A total of one to four individual determinations were averaged. These determinations were highly reproducible for the major proteins. TraSp was usually synthesised *in vitro* at a rate 10 to 15-fold slower than TraTp but the results were occasionally so variable that we present only the range of values. To normalise between chimaeric plasmids, the relative value of TraCp to TraJp was determined with pRS30-carrying minicells, and this value used to correlate further to TraFp and TraHp. The relative incorporation into TraJp (pRS27) and TraTp (pRS31) was averaged from individual determinations and used for normalising the results with TraSp and TraDp.

¶Two autoradiograms, one of which is shown in Fig. 4, were evaluated by densitometry to estimate the percentage of each *tra* protein associated with the cell envelope fraction. The values for TraCp and TraDp varied in the two experiments and the range rather than the average is presented.

exception of polypeptides B and B* (discussed below) the same additional protein species were detected regardless of the vector molecule. These additional proteins must therefore have been coded for by the cloned F DNA. Comparable results were also obtained with the other chimaeric plasmids shown in Fig. 1.

In an *in vitro* protein-synthesising system directed by purified plasmid DNA, only cistrons on f6 were expressed with equal efficiency from both pSC101 and ColE1Amp. The ColE1Amp-derived chimaeric plasmids pSH1 (*EcoRI* fragment f1) and pSH2 (f2) were poorer templates for protein synthesis *in vitro* than were the corresponding pSC101-derived plasmids pRS29 (f15, f1) and pRS31 (f17, f19, f2). We infer that the promoter adjacent to the *EcoRI* site in ColE1 is used more efficiently *in vivo* than in our *in vitro* system, and we interpret this as confirmation that the only strong promoter in the *tra* operon is on *EcoRI* fragment f6.

Isolation of *tra* mutant chimaeras

To assign individual proteins to particular *tra* genes, we isolated a series of *tra* mutants derived from pRS27, pRS29 and pRS31, each mutant carrying a defined point mutation which drastically altered the structure of the corresponding protein. We used two methods to introduce mutations into the chimaeric plasmids. Defined amber and UGA *tra* mutations were moved by homozygosity from *Flac* to the chimaeric plasmids. Alternatively, the chimaeric DNAs were treated *in vitro* with hydroxylamine¹¹ and mutants were detected after transformation¹² into an appropriate *E. coli* strain. Only amber or non-leaky mutations were analysed further. Mutants derived by both homozygosity and mutagenesis were detected and characterised by genetic complementation tests⁴. We succeeded in isolating at least two independent non-leaky mutations in each of fourteen *tra* cistrons (see Table 1). Each mutation affected the expression of only one known cistron. The collection included amber mutations in the *tra* cistrons *J*, *E*, *B*, *C*, *F*, *H*, *T* and *D*. No attempt was made to isolate *traG* mutants, since the only available chimaeric plasmid carrying an intact *traG* cistron, pRS26, was too unstable for genetic manipulation. We were unsuccessful in attempts to isolate

finP mutants of pRS27. All the mutant chimaeric plasmids were assigned numbers in the pBE series and some have already been described elsewhere^{4,6}.

Identification of conjugation proteins

Each assignment of a *tra* protein to a *tra* cistron reported here is based (with the exception of TraJp) on at least two independent mutations in that cistron resulting in the loss of, or change in a particular protein band. In essentially all cases, any one mutation had the same effect both *in vitro* and in minicells. Furthermore, there was a strict correlation between the cistron mutated and the protein affected, allowing us to identify proteins TraMp, TraJp, TraAp, TraLp, TraEp, TraBp, TraCp, TraFp, TraHp, TraSp, TraTp and TraDp. We now refer to these proteins as TraXp, rather than by our former nomenclature⁶ (pTraX), since the latter may be confused with designations for plasmid chimaeras such as pRS27. Representative results are presented in Fig. 3. The molecular weights of the proteins thus identified, and the numbers of mutations affecting those proteins are summarised in Table 1. The molecular weights of the *tra* proteins have been correlated with physical mapping data (refs 4, 13 and Thompson, personal communication) to produce the map in Fig. 1. Thus our results both identify and map twelve conjugation (*tra*) proteins. Of the genetically defined cistrons on F which are involved in conjugation, only the products of *traK*, *G*, *I* and *finP* remain unidentified. We also detected other proteins encoded by the F factor for which no genes are yet known. The four major unassigned protein species (three encoded by *EcoRI* fragment 6 and labelled 6a, 6b and 6c and one encoded by fragment 2 and labelled 2a) are indicated in Fig. 3. Numerous other protein species encoded by *EcoRI* fragment 2 were also detected (see Fig. 3).

Saturation of the map

In the following discussion we assume that *tra* cistrons do not overlap, that the origin of DNA transfer, *oriT*¹⁴, does not map

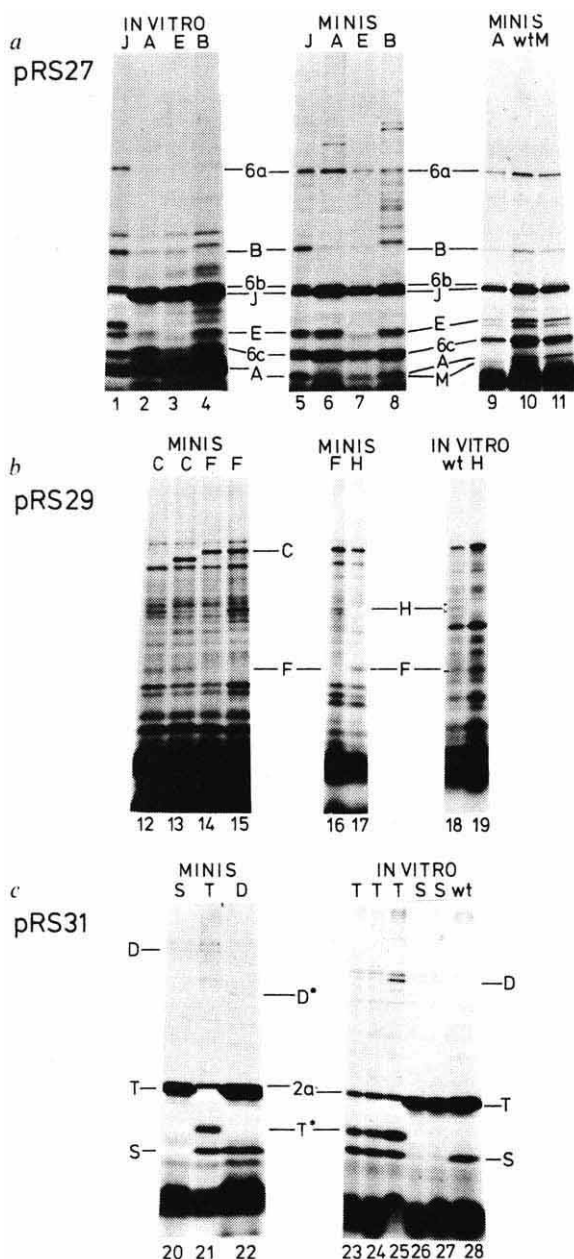


Fig. 3 Identification of *tra* proteins. SDS polyacrylamide gel analysis of proteins synthesised from plasmids pRS27 (a), pRS29 (b) and pRS31 (c) carrying mutant cistrons as indicated above each gel track (wt, wild type). Minicell proteins (minis) were prepared as in Fig. 2. *In vitro* protein synthesis was as in ref. 18 but purified initiation factors were used. The *in vitro* system was prepared from the $Su^- F^-$ strain JC3272 (ref. 2) and the incubation was carried out in 50 μ l volumes containing 250 μ g washed ribosomes, 2 μ g IF1, 4 μ g IF2, 0.5 μ g IF3, 2.5 μ g chimaeric plasmid DNA, 10 μ l of S-150 fraction, and the other components described (in ref. 18). After 45 min incubation at 37 °C, the samples were treated with 50 μ g ml $^{-1}$ each of DNase I and RNase I at 37 °C for 1 min. 50 μ l of twice concentrated sample buffer²⁴ was added and the samples processed for SDS gel electrophoresis and fluorography as described^{6,27}. Supercoiled plasmid DNA was purified from Triton X-100 cleared lysates²⁸ by CsCl-ethidium bromide centrifugation. After extracting the ethidium bromide into CsCl-saturated propan-2-ol, 30 to 70 μ g of DNA was concentrated into 100 μ l of buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8) by ethanol precipitation. The figure shows a representative selection of mutations which led to the loss of the encoded gene product, as follows (*Designates amber-suppressible mutations.): a, tracks 1,5, *traJ90**; tracks 2,6,9, *traA1*; tracks 3,7, *traE266**; tracks 4,8, *traB269*; track 11, *traM228*. b, track 12, *traC271**; track 13, *traC272**; tracks 14,16, *traF13**; track 15, *traF273**; tracks 17,19, *traH88**. c, tracks 20,26, *traS231*; track 27, *traS232*; track 23, *traT246**; tracks 21,24, *traT247**; track 25, *T248**; track 22, *traD14**. The following *tra* mutations also led to loss of the encoded protein: in pRS27, *A262*, *A278*, *E264**, *E281**, *B270**, *L263* and *L311*; in pRS29, *C5**, *F274**, *H282**, and *H283**; in pRS31, *S233*, and *D285*. C*, T*, and D*, amber peptides of TraCp, TraTp and TraDp.

within a cistron, and that the average molecular weight of an amino acid is 110. The *tra* cistrons to which we have assigned cistrons all lie in the 31-kb region on F between the 62 and 93 kb coordinates^{4,13}, and thus account for 33% of the coding capacity of this region. There is, however, a wide variation in the degree of saturation of the different *EcoRI* fragments. Thus the region on f6 to the right of *oriT* is almost completely saturated. In fact the space between *oriT* and a restriction endonuclease site in or near *traE* at 64.7 kb (R. Thompson, personal communication) is only sufficient to code for the defined proteins plus additional protein(s) of less than 20,000 daltons. This region includes the *finP* cistron¹⁵. Similarly, the available DNA between *traE* and *traB* requires that TraKp be smaller than 91,500 daltons. In contrast, the rest of the *tra* operon, on fragments 15, 1, 17, 19 and 2, includes large gaps with no known cistrons. Consequently the gene locations shown for this region in Fig. 1 are not nearly as well defined as those for f6.

Most *tra* products are membrane proteins

We have analysed the intracellular location of the *tra* proteins synthesised in minicells. The cell envelope was separated from the cytoplasm and both fractions were analysed by SDS gel electrophoresis. Most of the *tra* proteins proved to be strongly associated with the cell envelope (Fig. 4 and Table 1). The exceptions were TraCp and TraDp for which the proportions associated with the cell envelope varied widely between experiments. Although the possibility exists that the minicell results are not directly applicable to whole cells, we infer that the following phenomena are cell membrane related: the regulation of the *tra* operon by TraJp, the synthesis of F pili involving TraAp, TraEp, TraBp, TraFp and TraHp, and the poor recipient ability mediated by TraSp and TraTp.

Control by traJ

TraJp seems to be necessary for efficient transcription of the F factor *tra* operon in intact cells⁸. It is interesting that TraJp exhibited properties which are unusual for a control protein: it was associated with the cell envelope and it was synthesised at a very high rate. We anticipated that *traJ*⁻ mutants of pRS27 would exhibit pleiotropic reduction in the expression of the other *tra* operon proteins. This was not the case however. For four independent *traJ*⁻ mutants of pRS27 (one amber-suppressible (J90) (see Fig. 3, tracks 1,5) and three missense mutations), the rates of synthesis, *in vitro* and in minicells, of TraAp, TraEp and TraBp remained unchanged or even increased slightly. Thus whether TraJp is synthesised in a defective form, or is not synthesised at all, no regulatory effects on *tra* operon cistrons were detected. Since the *in vitro* system used may have been lacking in cell components involved in regulation, we attempted to reconstitute dependence on TraJp by adding cell-free extracts from F⁺ cells to the *in vitro* system. These attempts were unsuccessful. Furthermore, no differences attributable to control by *traJ* were found when pRS27 or pRS27 *traJ*⁻ DNA templates were expressed in cell-free systems prepared from F⁻, F⁺ *traJ*⁺, or F⁺ *traJ*⁻ cells. These negative results contrast with those obtained for several other genetic control proteins which do function *in vitro*¹⁶⁻¹⁸. Two possibilities could account for our results. If TraJp normally counteracts a mechanism which terminates transcription, the mechanism itself may be so labile that it is absent both *in vitro* and in minicells. Alternatively, TraJp might increase the binding specificity to the *tra* operon promoter of some cell component such as RNA polymerase. The absence of competing DNA (and promoters) *in vitro* and in minicells may then render this increased binding specificity superfluous.

F pili

There is evidence that *traA* codes for the F pilus subunit protein F pilin¹⁹. We found that TraAp had the following properties. In minicells, it was synthesised at a much slower rate than *in vitro*, where it was the major *tra* product (Table 1 and Fig. 3a). It had an apparent molecular weight of 13,700 (Table 1 and Fig. 3, tracks

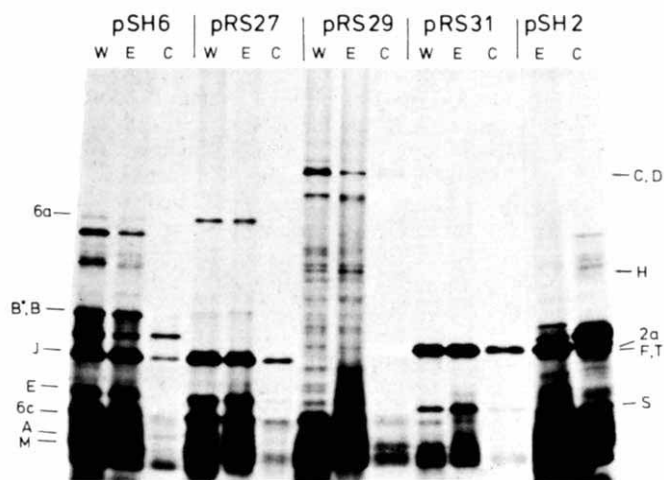


Fig. 4 Intracellular location of *tra* proteins. Minicells containing radioactively labelled, plasmid-coded proteins, were fractionated into envelope and cytoplasmic components. The autoradiogram shows proteins from whole minicells (W), envelope (E) and cytoplasmic (C) fractions. The relevant plasmids are indicated above the tracks. The proteins encoded by pSH6 and pRS27 are indicated on the left of the figure whereas those encoded by pRS29 (C,H,F), pRS31 (D,T,S) and pSH2 (D,T) are indicated on the right. A minicell suspension (1.5 ml) prepared as in Fig. 2 was treated with $50 \mu\text{g ml}^{-1}$ of lysozyme and 1 mM EDTA, $\text{pH } 7.5$ for 10 min at 37°C . The minicells were disrupted by sonication (six bursts of 30 s with cooling) and any unbroken minicells were removed by brief centrifugation. Minicell envelopes were pelleted at $48,000 \text{ g}$ for 60 min at 2°C . The cytoplasmic proteins in the supernatant were precipitated with trichloroacetic acid and resuspended in $50 \mu\text{l}$ of sample buffer²⁷. The membranes were resuspended in the same volume of sample buffer. Staining of the SDS gel before autoradiography revealed that distinctive cell envelope and cytoplasmic proteins were indeed enriched in the appropriate fractions.

9–11), and did not comigrate with F pilin obtained from purified F pili²⁰ (data not shown). F pilin has an apparent molecular weight of $10,700$ (ref. 20). Thus if *traA* does code for F pilin, TraAp may be a precursor which is processed by a specific protease in the cell, losing a $3,000$ dalton polypeptide to generate the F pilus subunit. However, this processing did not take place in minicells, where TraAp comigrated with the *in vitro traA* product and was associated with the cell envelope. We were unable to precipitate TraAp from *in vitro* reaction mixtures by purified F pilus antibodies (data not shown). It therefore remains possible that TraAp does not represent a precursor for F pilin, and that F pilin is coded for by a cistron other than *traA*.

Map position of *traB*

Genetic complementation has shown that biologically active TraBp is synthesised both by pRS27 (carrying f6 and f15) and by pSH6 (carrying only f6)⁴. This leads to the conclusion that all of *traB* is located on f6. However, we found that no protein coded for by pSH6 comigrated with TraBp (Fig. 2). A new band, $1,000$ – $2,000$ daltons larger appeared instead (B* in Fig. 2). We speculate that the last few carboxyterminal amino acids of TraBp are not essential for its biological function, and that the *traB* cistron possesses an *EcoRI* site very near its end. The additional $2,000$ – $3,000$ daltons in B* (the TraBp analogue encoded by pSH6) would then be derived from a vector gene, probably that coding for colicin. The behaviour of one of the *traB* mutants supports this interpretation. We isolated an amber-suppressible mutant of pRS27 (carrying *traB270*). This coded for a TraBp amber peptide approximately five amino acids shorter than intact TraBp. This polypeptide retained 40% of its ability to complement *traB* mutants of the F sex factor, indicating that the carboxyterminal end of TraBp is not very important for its biological function. We have taken account of these considerations in locating *traB* as shown in Fig. 1.

Surface exclusion

The poor recipient ability of F-carrying cells in conjugation with other F-carrying donor cells, is coded for by *traS* and *traT* (refs 5,6 and M. A., R. Thompson, S. Schwuchow, B. Kusecek and N. Willetts, manuscript in preparation). TraTp is an outer membrane protein present in up to $85,000$ copies per cell⁶. This is of the same order of magnitude as that of the major *E. coli* outer membrane proteins I and II*. It blocks the conversion of mating aggregates from an unstable to a stable form^{5,6}. TraSp markedly reduces DNA transfer even within stable mating aggregates⁶.

The results presented here demonstrate that TraTp and TraSp are incorporated into the cell envelopes of minicells, that TraTp is one of the major products of the *tra* region and that TraSp is synthesised at widely varying rates relative to TraTp. These data also allow us to make a preliminary genetic mapping of *traS* and *traT*. The chimaeric plasmid pRS31 carries *EcoRI* fragments 17, 19 and 2 and directs the synthesis of both TraSp and TraTp (Fig. 2, track 8). The chimaeric plasmid pSH2 carries only *EcoRI* fragment 2 and codes for TraTp but not TraSp (Fig. 2, track 5). Thus while *traT* maps entirely or almost entirely on f2, *traS* probably maps at least partially on f17 or f19 as drawn in Fig. 1.

Post-transcriptional regulation

There was great variation in the net rate of synthesis of individual *tra* operon proteins. Those with much the highest rate were TraAp, which was the main *in vitro* product, and TraTp. TraTp was synthesised at very high rates, both *in vitro* and in minicells, and it was the only *tra* operon product detectable on SDS gel electrophoresis of whole cell envelope proteins⁶. The cistrons coding for these two proteins are located at the beginning (*traA*) and towards the end (*traT*) of the *tra* operon (Fig. 1).

In contrast other proteins coded for by all three of the major *EcoRI* fragments of the *tra* region were synthesised at low rates. These include TraMp, TraEp, TraBp, TraCp, TraFp, TraHp, TraDp and the numerous unidentified proteins mainly coded for by fragment f1. All these proteins, except TraMp, are coded for by cistrons within the *tra* operon. They are always synthesised at low rates, irrespective of the vector or the size of the cloned DNA directing their synthesis. Comparable results for the same proteins were obtained whether transcription proceeded from the *tra* operon promoter (pRS27, pSH6 and pRS30) or from a promoter on the plasmid vector (pRS26, pSH1, pSH2 and pRS31). Thus cistrons transcribed after *traA* and before *traT* were expressed much less efficiently than *traA* and *traT* themselves (Table 1). Polar Mu-1 insertions in the *tra* operon have demonstrated that it contains no strong secondary promoters. Thus the increased rate of synthesis of TraTp relative to the gene products of promoter proximal cistrons must result from regulation occurring at the post-transcriptional level. Since this control was not dependent on any particular promoter, we infer that the rate of translation is determined by the nucleotide sequence of the primary transcript. This conclusion adds another case to the list (see refs 21–23 for example) of recently reported examples of post-transcriptional rate determination in bacteria.

Conclusions

Previous genetic analyses of *tra* cistrons on the F sex factor led to the present, relatively advanced, understanding of their regulation and biological function⁵, but yielded no information on the proteins involved. The genetic results are now complemented by the identification presented here of 12 of the *tra* cistron gene products. These results have facilitated the construction of a physical map showing the size and location of known *tra* cistrons, they have indicated the existence of numerous unidentified *tra* cistrons and proteins, and they have enabled us to begin an investigation at the biochemical level into the roles of these proteins in bacterial conjugation.

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letters to nature

Superfluid $^3\text{He A}$ is a liquid ferromagnet

THE superfluid A phase of liquid ^3He (see for example refs 1, 2) is often said to display orbital ferromagnetism³. This term generally seems to be meant metaphorically and to imply that this phase possesses a finite total orbital angular momentum in equilibrium³. I point out here that superfluid $^3\text{He A}$ is in fact quite literally ferromagnetic, with a spontaneous magnetic moment which, although extremely small on the scale of ordinary ferromagnets ($\sim 10^{-11}$ Bohr magnetons per atom) is probably measurable and may indeed have some interesting experimental implications.

The basic mechanism is that the Cooper pairs in $^3\text{He A}$ (which for present purposes may be thought of as giant diatomic molecules) possess a relative orbital angular momentum³ of $\hbar$ directed along the so-called **I** vector²; in a way familiar in the theory of diatomic molecules⁴, a small part of this is transferred, via the electronic-rotational coupling, into motion of the electrons around the nuclei, thereby producing a finite magnetic moment for each pair. In contrast to the thermally disoriented diatomic molecules in an ordinary gas, however, all the Cooper pairs in $^3\text{He A}$ have the same direction of orbital angular momentum, so the system behaves as a ferromagnet.

I have attempted to calculate the order of magnitude of the spontaneous magnetic moment so produced by a technique closely analogous to that used in ref. 5 to estimate the parity-violating electric dipole moment of $^3\text{He B}$; that is, I first calculate the magnitude $\mu(R)$ of the orbital magnetic moment of a single ^3He dimer with nuclear separation R and relative orbital angular momentum $\hbar$, then multiply by the square of the radial part of the Cooper-pair wave function, $|F(R)|^2$, and integrate over all R . (The average over the angular wave function gives unity.) For $F(R)$ I make the same ansatz as in ref. 5, namely $F(R) = AR^{-1} \sin k(R-R_0)$, where R_0 is the 'hard-core radius' ($\sim 2.5 \text{ \AA}$), A is fitted from the experimental dipole energy² $g_D(T)$ and I assume $k \gtrsim k_F$ (k_F = Fermi wavevector). This gives for the spontaneous magnetic moment per unit volume along **I** in a uniform sample

$$M(T) \cong \frac{g_D(T)R_0^2}{\gamma^2 \hbar^2} \int_{R_0}^{\infty} \mu(R) \cdot 2 \sin^2 k(R-R_0) dR \quad (1)$$

To estimate the quantity $\mu(R)$ I first note that if a single neutral atom of mass M , in a slightly deformed electronic S-state, is constrained to rotate about the origin with its nucleus at a distance $R/2$ and with angular momentum $\hbar/2$, then the orbital magnetic moment is given, to lowest order in the deformation, by the expression $-2R^{-1}P(R)(m/M)|\mu_B|$, where m is the electron mass, μ_B the Bohr magneton and $P(R)$ the polarisation of the atom in the outward direction in units of e ($\equiv -|e|$). I shall now assume that for a ^3He dimer with nuclear separation R and relative angular momentum $\hbar$ the correct order of magnitude, at least, of $\mu(R)$ may be obtained by approximating the electronic wave function by an antisymmetrised product of $1\sigma_g$ and $1\sigma_u$ SCF-MO single-particle functions as in for example ref. 6, calculating $P(R)$ for each atom separately and summing over the two atoms. For the SCF-MO functions I use the forms computed numerically in ref. 6. Since there are only four relevant data points and the corresponding values of μ by no means fall on a smooth curve, any attempt to determine the general form of $\mu(R)$ in this way is somewhat hazardous, but if we arbitrarily try to fit it to the intuitively plausible formula (a_0 = Bohr radius)

$$\mu(R) = |\mu_B| \left(\frac{m}{M} \right) \left(\frac{a_0}{R} \right) C_0 \exp\{-\lambda(R-R_0)/a_0\} \quad (2)$$

then the 'best' values are probably $C_0 \cong -1.1 \times 10^{-3}$, $\lambda \cong 1.4$.

It is convenient to express the final result for the spontaneous moment $\mathbf{M}(T)$ in terms of an equivalent field $\mathbf{H}_{eq}(T) \equiv \mathbf{M}(T)/\chi_p$, where χ_p is the normal-state paramagnetic susceptibility (not the total susceptibility). Note that 1 G is equivalent to about $5 \times 10^{-10} \mu_B$ per atom. Combining equations (1) and (2) and substituting the experimental value² of $g_D(T)$ for pressures near the melting curve and $T/T_c \gtrsim 0.7$ (T_c = critical temperature of $^3\text{He A}$) we find in this region

$$\mathbf{H}_{eq}(T) = H_1(1 - T/T_c)\mathbf{I}, \quad H_1 \cong -0.02 F(k) \text{ G} \quad (3)$$

where $F(k) \cong (1 + 2k_F^2/k^2)^{-1}$. Thus I tentatively estimate $|H_1| \sim 10\text{--}20 \text{ mG}$; however, in view of the uncertainties in both the chemical and the many-body parts of the calculation this should be regarded as at best a crude order of magnitude.

A spontaneous magnetisation of this order should be detectable, probably most easily by observing its effect on the orientation of the **I**-vector (as measured, for example, by ultrasonic

attenuation) at either low or ultra-high external fields (J. C. Wheatley, D. M. Lee, personal communications). (Note that unlike previously known orientation effects, this one is sensitive to the polarity of the field.) Its observation would have a number of interesting implications. (1) It would provide the first direct evidence that invariance under both space inversion and time reversal is spontaneously broken in $^3\text{He A}$. (2) The observed order of magnitude would provide some measure of the credibility of the above method of calculating 'chemical' effects in superfluid ^3He , and hence for example of the theoretical predictions of ref. 5. (3) There should be related effects in $^3\text{He B}$, for example, an interaction of the nuclear spins with the electronic orbital moments⁷ which will shift the spin-orbit rotation angle² slightly away from the 'magic angle' $\cos^{-1}(-1/4)$. (4) $^3\text{He A}$ would presumably constitute the first ferromagnetic liquid known in nature.

I thank Bill Truscott (who first suggested to me the possibility of orbital magnetic effects in superfluid ^3He), John Wheatley, Richard Webb and Dave Lee for helpful discussions, and Kathy Levin and her colleagues in the Solid State Theory group at the James Franck Institute for their hospitality.

A. J. LEGGETT

James Franck Institute, University of Chicago
and

School of Mathematical and Physical Sciences,
University of Sussex, Brighton, UK*

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*Permanent address.

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Positions of galactic X-ray sources: $0^\circ < l^\text{II} < 20^\circ$

PRECISE (20–25'') positions of six X-ray sources located in the galactic bulge, GX1+4, GX9+9, GX3+1, GX1, G%13+1 and GX17+2 are reported here. The data were taken as part of the survey of the galactic plane performed with the SAS-3 rotating modulation collimators^{1–4}. Previously proposed optical counterparts for three of these sources (GX1+4, GX9+9, and GX17+2) lie within our error circles. The positions, error radii, and

intensities (2–11 keV) determined for the sources are given in Table 1. We compare our results with those determined with previous sounding rocket and satellite experiments in Fig. 1. Proposed optical and radio candidates are also included. Finding charts for the six sources are given in Fig. 2.

The status of each source with regards to optical or radio identifications is as follows:

GX1+4 (2S1728–247): A candidate for this source was suggested by Glass and Feast⁵, who detected it first in the infrared (1.25–2.2 μm) and then optically, where nearly all the emission is in lines, primarily H α . The unusual nature of this object has been verified in subsequent work^{6,7}, and its existence in the small Copernicus (K. Mason, personal communication in ref. 6) and SAS-3 X-ray error boxes leaves little doubt about the identification.

GX9+9 (2S1728–169): An optical candidate for this source has been suggested by Davidsen, Malina, and Bowyer⁸ on the basis of the Copernicus⁹ and Uhuru⁹ positions (see Fig. 1). It has a B magnitude of 16.4 with an ultraviolet excess ($U-B = -0.5$). The object lies 10'' from the position determined with the SAS-3 RMC, well within the error circle. Recently, emission lines of He II $\lambda 4686$ and the C III, N III blend at 4640–4650 have been observed¹⁰ in this candidate. The substantial reduction in X-ray error box area and the detection of emission lines common in optical counterparts of X-ray sources make the identification certain.

GX3+1 (2S1744–265): A very precise position for this source has been determined with a pair of lunar occultations¹¹. The centre of our error circle is within 3'' of the centroid of the occultation determination. A weak radio source (G. K. Miley, personal communication in ref. 11) lies near the occultation position and is consistent with the SAS-3 position. There are no optical candidates for the source^{11,12}.

GX9+1 (2S1758–205): There are numerous position determinations for this source^{8,9,13–15} (see Fig. 1) but no optical candidates^{12,13,16}. A weak radio source lies nearby¹⁷, but is excluded as a candidate by the X-ray positions.

GX13+1 (2S1811–171): There are currently no optical or radio candidates for this source, although searches have been made in both wavelength bands^{6,18}.

GX17+2 (2S1813–140): A variable radio source was found by Hjellming and Wade¹⁹ in the early X-ray error

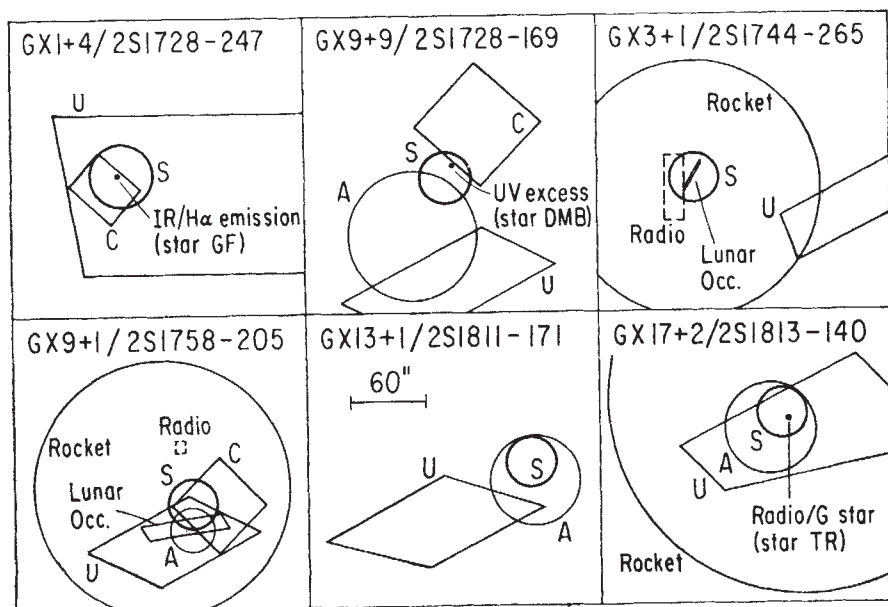
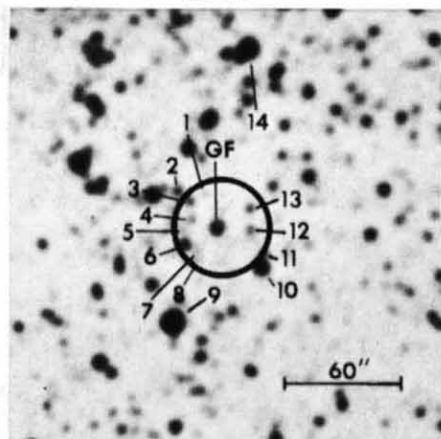
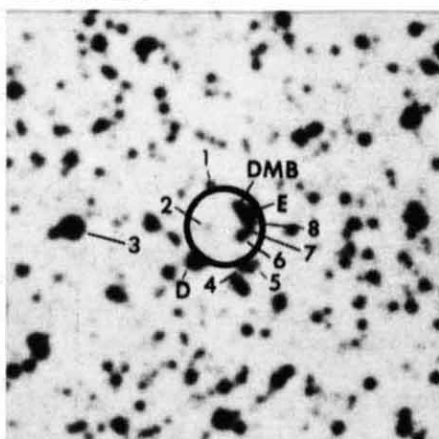


Fig. 1 The regions containing X-ray sources reported in this letter. The indicated scale factor applies to all regions. Optical and radio candidates are discussed in the text. X-ray error boxes are from the SAS-3 (S, this work); Uhuru (U, ref. 27); Ariel V (A, ref. 14), and Copernicus (C, ref. 8, and K. Mason, personal communication in ref. 6) satellites and from lunar occultations^{11,13} and rocket¹⁵ experiments.

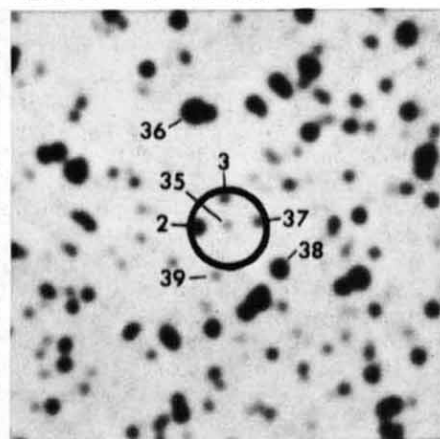
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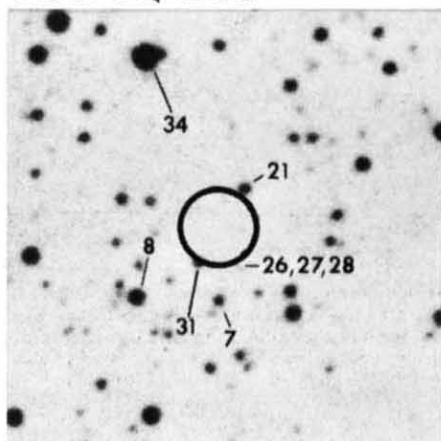
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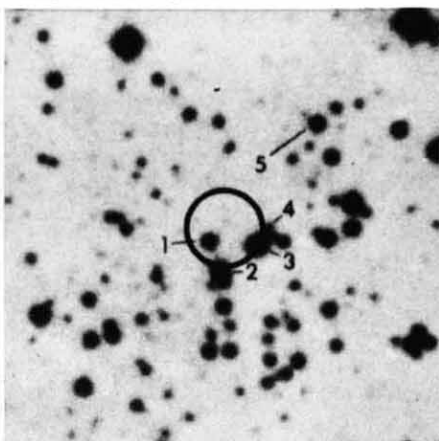
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2S1758-205



2S1811-171



2S1813-140

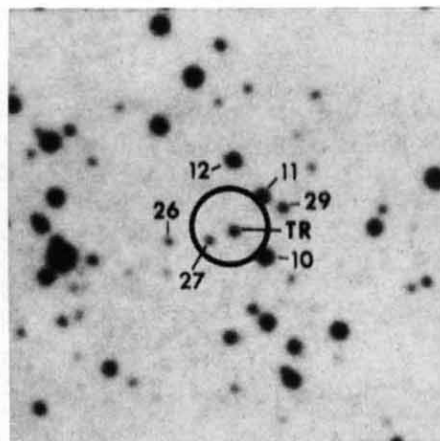


Fig. 2 Finding charts for the SAS-3 positions. The charts are taken from the Palomar Sky Survey red plates (National Geographic Society). North is up and east is to the left. The indicated scale factor applies to all charts. Stars are numbered to ease communication between observers; numbering and lettering schemes of previous observers have been adopted. The proposed identifications of Glass and Feast (GF, ref. 5), Davidsen, Malina, and Bowyer (DMB, ref. 6) and Tarenghi and Reina (TR, ref. 21) are indicated.

Table 1 Celestial positions

SAS-3 designation	Other designations*	Position (1950)		l^{II} b^{II}	Error radius (90%)	Flux density† (2–11 keV)	Comments‡
		α	δ				
2S1728-247	GX1+4 GX2+5 4U1728-24	17 h 28 min 57.4 s 262.2392	-24° 42' 42'' -24.7117	1.9 4.8	25''	55 μJy	Optical counterpart
2S1728-169	GX9+9 4U1728-16	17 28 50.4 262.2100	-16 55 30 -16.9250	8.5 9.0	20	230	Optical counterpart
2S1744-265	GX3+1 4U1744-26	17 44 49.0 266.2042	-26 32 51 -26.5475	2.3 0.8	20	400	Possible radio candidate
2S1758-205	GX9+1 4U1758-20	17 58 33.3 269.6388	-20 31 44 -20.5289	9.1 1.2	20	480	
2S1811-171	GX13+1 4U1811-17	18 11 37.2 272.9050	-17 10 16 -17.1711	13.5 0.1	20	325	
2S1813-140	GX17+2 4U1813-14	18 13 11.2 273.2967	-14 03 10 -14.0528	16.4 1.3	20	490	Radio/optical candidate

*Refs 25–28.

†Averaged over the observation and accurate to $\sim 10\%$. 1.0 μJy corresponds to $2.2 \times 10^{-11} \text{ erg s}^{-1} \text{ cm}^{-2}$ (2–11 keV). $I_{\text{crab}} = 1,060 \mu\text{Jy}$ (see refs 1, 2).

‡See text for discussion and references.

boxes^{15,20}. Tarengi and Reina²¹ pointed out that there is only one star visible on the Palomar Sky Survey within the radio error box. Hoag and Weisberg²² made a deep red survey of the region and found one additional faint M star which lies in the SAS-3 error circle, but none in the radio error box to a limit of $R \sim 20$ mag. The identification with the Tarengi and Reina candidate depends on the assumption that the radio and X-ray sources are coincident. The optical object has a normal G type spectrum⁶ and shows no evidence²³ of the reported 30 min X-ray period²⁴.

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R. E. DOXSEY
K. M. V. APPARAO*
H. V. BRADT
R. G. DOWER
J. G. JERNIGAN

Department of Physics and Center
for Space Research,
Massachusetts Institute of Technology,
Cambridge, Massachusetts 02139

Received 12 September; accepted 24 October 1977.

*Permanent address: Tata Institute of Fundamental Research, Bombay, India.

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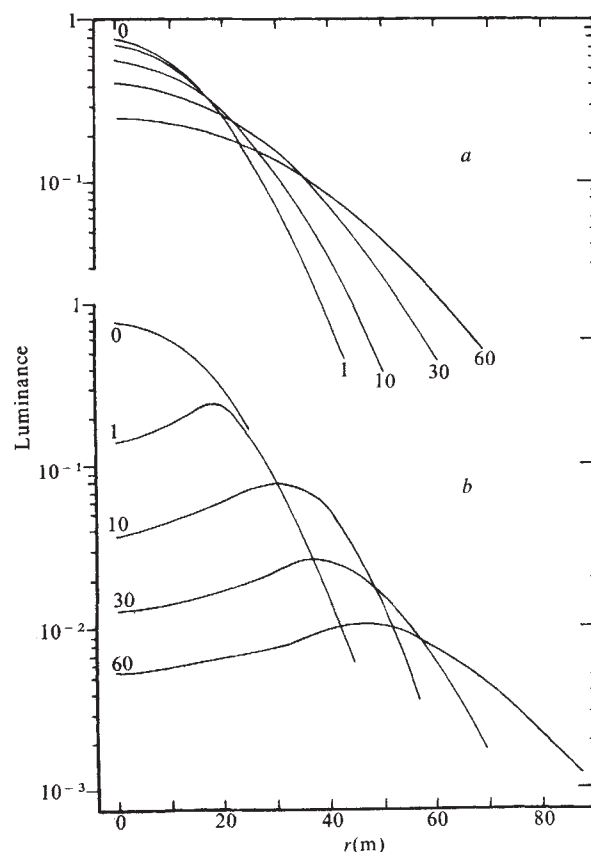


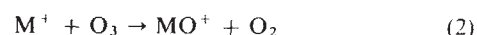
Fig. 1 Train surface brightness profiles for meteors of 30 and 60 km s⁻¹ at various times (s) after train formation; r is distance from train axis. Ordinate is normalised luminance. Solutions refer to meteoroid mass of 30 g zenith distance 30° corresponding to meteors of visual magnitudes approximately -3 and -6 at (a) 30 and (b) 60 km s⁻¹ respectively.

models of the mechanism of long enduring train luminosity.

An ablating meteoroid deposits Na atoms (meteoric Na abundance $\sim 0.7\%$ see ref. 6) in a column some tens of kilometres long and a few metres in diameter. As a consequence of ozone association followed by sodium oxide reduction in the presence of atomic oxygen, production of excited Na atoms in the (²P) state occurs followed by emission of the well known D lines. On a simple model ground state Na (²S) atoms are continually recycled acting as a catalytic agent. A meteor of fireball magnitude should deposit sufficient sodium to produce a visible enduring train⁵. It is possible, however, that under certain circumstances removal of Na atoms may occur, a process being permanent loss by charge exchange. Although the night-time concentrations of atmospheric positive ions in the upper D region are too low to be effective, meteoric metal ions present in the diffusing meteor column may be significant in transferring charge to free Na atoms



where M^+ represents metal ions ($M = Fe, Mg, Si$). The most abundant meteoric ions O^+ are rapidly lost by charge exchange with the major atmospheric neutrals rather than by equation (1). This reaction represents a permanent loss of Na as radiative recombination or a sequence of reactions involving the major atmospheric neutrals results in negligible re-emergence of Na atoms. Metal ions themselves can suffer loss by association with atmospheric neutrals the most important route at 85 km is



giving metal oxide ions which dissociatively recombine with

Hollow meteor trains

ENDURING meteor trains that appear at a height of about 85 km as double lines of light have been reported for more than a century, the first record being Newton's¹ in 1869. Although the dual appearance, which has been seen in the telescope within a few seconds of train formation and by the naked eye after about 1 min, is not due to the occurrence of separate trains², no explanation has yet been offered for the phenomenon. The peculiar formation almost certainly results from the expanding meteor train assuming the shape of a hollow cylinder³ and it is shown here that this behaviour is to be expected on the basis of recent^{4,5}

electrons rapidly and at much faster rate than reduction by atmospheric O to restore M^+ .

There are two significant factors in determining the spatial distribution of species in the expanding train. First, because of the relatively large extent of their coulomb fields, positive ions possess a smaller momentum transfer mean free path than neutral atoms, the radius of the cylindrical ion column at train formation is expected to be less than that of the neutral atom column. Second, the diffusion coefficient of free sodium atoms in atmospheric gases is several times greater than that of positive meteoric ions (which diffuse at the ambipolar rate). Consequently as the atomic Na and M^+ columns have different radii throughout their expansions a proportionately greater loss rate of Na near the column axis due to equation (1) results. Because Na atoms are mopped-up much faster in the central regions of the meteor train a trough may develop in which relatively few Na (^{24}P) atoms are produced. In addition, by equation (2) ozone molecules are depleted⁷ much faster in the central regions of the column, so that fewer O_3 molecules are available to take part in the catalytic cycle with Na which leads to the production of Na (^{24}P). There are, therefore, two processes leading to a relatively low concentration of excited sodium atoms in the central regions of an expanding train. The depth and lifetime of the trough is determined by the speed of the chemistry, equations (1) and (2), compared with the effects of diffusion in restoring the equilibrium radial profile by transporting Na and O_3 inwards and eliminating the hollow. It is also significant that the central hollow will only develop if the total number of M^+ ions in a cross section of the train, α_{M^+} , greatly exceeds the number of Na atoms, α_{Na} . As the ionisation probability, β , for ionising collisions between meteoric atoms and atmospheric neutrals increases rapidly with increasing meteoroid velocity, the hollow meteor train is expected to be a high velocity phenomenon.

Early in the life of a train when molecular rather than eddy diffusion governs the radial expansion, the loss of catalytic sodium may be described essentially by the equations:

$$\frac{\partial}{\partial t}[\text{Na}] = D_{\text{Na}} \nabla^2[\text{Na}] - \Sigma k_1[\text{Na}][M^+]$$

$$\frac{\partial}{\partial t}[M^+] = D_a \nabla^2[M^+] - k_1[\text{Na}][M^+] - k_{2M^+}[\text{O}_3][M^+]$$

$$\frac{\partial}{\partial t}[\text{O}_3] = D_{\text{O}_3} \nabla^2[\text{O}_3] - \Sigma k_{2M^+}[\text{O}_3][M^+]$$

where D is the appropriate diffusion coefficient, ∇^2 the Laplacian in cylindrical coordinates and the number densities of various species are represented by their appropriate species symbol. Numerical solutions were obtained for a height of 85 km taking night-time $[\text{O}_3] = 8 \times 10^8 \text{ cm}^{-3}$. The adopted rate coefficients ($\text{cm}^3 \text{ s}^{-1}$) are (ref. 8) $k_1 = 2.4 \times 10^{-9}$ (independent of the positive ion), $k_{2\text{Fe}^+} = 1.5 \times 10^{-10}$, $k_{2\text{Mg}^+} = 2.3 \times 10^{-10}$ and $k_{2\text{Si}^+} = 1.0 \times 10^{-10}$ (estimate). The diffusion coefficient ($\text{cm}^2 \text{ s}^{-1}$) of sodium atoms in air is a factor of ~ 3 greater than ambipolar⁹ and at 85 km with $T = 185 \text{ K}$ we adopt $D_{\text{Na}} = 4.2 \times 10^4$ and $D_a = 1.4 \times 10^4$. A value of $D_{\text{O}_3} = 4 \times 10^4$ is assumed. It is further assumed that following the ablation process, a meteor column is established whose constituent species have gaussian radial distributions described by radii r_i such that $r_i^2 \propto D$. For a meteor of fireball magnitude we take r_i (Na) = 20m. The results of Sida¹⁰ are used for a working model giving β for meteoric atoms as a function of velocity while atom abundances appropriate to chondrite meteorites are adopted. Visual meteor magnitude, meteoroid pre-entry mass and velocity may be related^{11,12} the line densities, α , of deposited atoms and ions. The ratios $\alpha_{M^+} \alpha_{\text{Na}}$ for 30 km s^{-1} and 60 km s^{-1} are 0.26 and 3.9 respectively. The sodium D line emission per unit volume is equal to the rate of production of Na (^{24}P) atoms so that for an optically thin column the apparent surface brightness (luminance) of any part of the train is proportional to the integral of the

product of the Na and O_3 concentrations in a column of unit area along the line of sight. Because β is highly velocity dependent, significant Na loss occurs only in trains of high velocity meteors. A central hollow only develops for $v > 50 \text{ km s}^{-1}$. Solutions are shown in Fig. 1 where the peak to trough luminance ratio is about 2 at 60 km s^{-1} while at 70 km s^{-1} is about 3.5. These ratios result from severe depletion of Na (^{24}P) atoms within a cylinder of radius about 20 m. A hollow train 60s after formation would be resolved by the naked eye at a distance of about 150 km. Such characteristics are in accord with observations. Although hollow train observations are sparse, the cases reported by Newton¹ refer exclusively to members of the Leonid shower (72 km s^{-1}) while those by Trowbridge² occurred at the epochs of the Orionids (66 km s^{-1}) and Perseids (60 km s^{-1}). The phenomenon has not been reported for the low velocity streams such as the abundant Geminids (36) Giacobinids (23) and Quadrantids (40 km s^{-1}).

W. J. BAGGALEY

Physics Department,
University of Canterbury,
Christchurch, New Zealand

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Surface temperature of early Earth

CHANGES in the surface temperature of the Earth throughout its history are important for understanding both the geological development of the Earth's surface and the origin and development of life on Earth. The calculation of variations in the surface temperature with time is complicated by the number and nature of the physical variables involved, as well as by the long period of time over which extrapolation is required. Theoretical studies must be carried out in terms of highly simplified global atmospheric models with fairly lax boundary conditions. Consequently, even the gross features of atmospheric evolution may be explicable in terms of more than one theoretical model. Here we take the model most widely referred to in recent years and point out that a radically different approach can provide an equally supportable result. Too much significance should not, therefore, be read into the details of currently constructable models.

Sagan and Mullen¹ carried out a theoretical investigation of long-term changes in the Earth's surface temperature on the assumption that the major infrared absorbing gases in the Earth's atmosphere have always been water vapour and carbon dioxide. But, in view of the accepted boundary conditions for the early Earth, they concluded that the original terrestrial atmosphere must also have contained additional absorbing gases. This conclusion has two important consequences. First, the Earth's early atmosphere must have undergone a significant change in chemical composition as the postulated additional absorbing agency was removed from circulation. Second, because any physically probable additional absorber is likely to belong to a chemical species that figures in current discussions concerning the origin of life (Sagan and Mullen consider ammonia to be the most probable candidate) acceptance of their model has implications for ideas on the early development of life on Earth. We intend to demonstrate here that the same boundary conditions can be satisfied by a different model that postulates absorption by water vapour and carbon dioxide only throughout the lifetime of the Earth.

In principle, the surface temperature at any epoch can be

calculated in two stages. The first involves the computation of the effective temperature of the planet (T_e) by the equation

$$S(1 - A) = f\sigma T_e^4 \quad (1)$$

where S is the solar constant; A the spherical albedo of the Earth; f the flux factor; σ the Stefan-Boltzmann constant. All the factors are straightforward except for f , which we will now define. For a rapidly rotating planet with a thick atmosphere, the area emitting radiation is taken to be $4\pi R^2$ (where R is the planetary radius). For a slowly rotating planet with a thin atmosphere, the corresponding emitting area is $2\pi R^2$. Since the area receiving solar radiation is πR^2 , the flux factor (defined as the ratio of the emitting area to the absorbing area) must, therefore, be either 4 or 2. But the choice need not always be so obvious. In physical terms, the value assigned to the flux factor depends on the ratio of two characteristic times. The first (τ_h) is the time required for a planetary atmosphere to radiate away a major part of its heat content: the second is the rotational period of the planet (τ_r). If $\tau_h/\tau_r \approx 1$, neither $f = 4$, nor $f = 2$, represent good approximations, and it becomes necessary to use a transitional value (for a more detailed discussion of f see ref. 2).

The second stage of the calculation relates T_e to the surface temperature (T_s) by an equation of the general form

$$T_s = T_e + \Delta T \quad (2)$$

(Here ΔT is the 'greenhouse' increment due to the presence of an atmosphere, and depends on the mass, surface pressure and chemical composition of the atmosphere concerned.) We have, therefore, set up a computer program that solves equations (1) and (2) for T_s over a wide range of the relevant input parameters and at any epoch. Our approach is similar to that of Sagan and Mullen, though we have incorporated a reanalysis of the laboratory data available on infrared absorption as a function of pressure (for further details, see refs 2 and 3).

A determination of changes in the terrestrial surface temperature entails a study of all possible secular variations in the input parameters over the lifetime of the Earth (taken to be $4.5\text{--}4.6 \times 10^3$ Myr). The best attested change is in the solar constant. There is good agreement between theoretical models of the Sun, showing that S has increased by 40–45% since the origin of the Solar System⁴. If this conclusion is fed into the model computations for T_s , then curve Fig. 1*b* results, giving a surface temperature well below the freezing point of water during the early part of the Earth's history. There is, however, an important observational limitation on the minimum surface temperature of the early Earth. Geological evidence suggests the presence of extended sheets of liquid water on the Earth's surface at least 3,700 Myr ago⁵. We cannot use this as an index of the degassing rate, as the actual volume of water cannot be readily determined,

but we can assert that the average surface temperature could not at that time have been much below 273 K.

Sagan and Mullen escape from this dilemma by suggesting that the early terrestrial atmosphere contained an additional absorbing agency. They thus increase ΔT until the required minimum value of T_s is reached. But the same final value of T_s might be obtained by increasing T_e . If a smaller value of S is accepted, then it can be compensated, according to equation (1), by making A larger, f smaller, or by changing both simultaneously. We investigate here whether such changes can provide an acceptable alternative model.

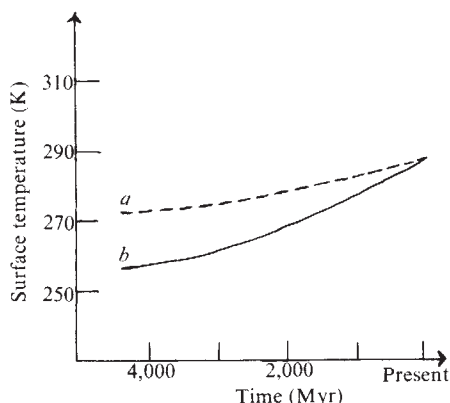
Sagan and Mullen assumed that the terrestrial albedo has always been approximately the same; but this assumption depends on the precise mode of formation of the atmosphere. It is generally supposed that the Earth's atmosphere (together with the hydrosphere and some of the sedimentary material) originated by degassing from the crust. If the atmospheric gases were released over an extended period of time, we would expect the albedo to change with time—from an initial value appropriate to an atmosphereless planet (say, 0.07) to the present-day value of 0.33. We have computed the run of the surface temperatures for a model where the gases forming the terrestrial atmosphere have been vented throughout the planet's existence. The terrestrial albedo has been taken to be a function of the mass of atmospheric gases per unit area. This approximation depends on the assumption that the planetary albedo is primarily affected by the presence of clouds, which can, to some extent, be correlated with atmospheric mass.

The results are shown in Fig. 1*a*. A comparison of the two curves in Fig. 1 indicates that the variation in albedo partially compensates for the change in the solar constant. Consequently, a relatively slow degassing leads to an average surface temperature that never falls far below the freezing point of water.

So far we have considered changes in the albedo due to continuous degassing. Available K/Ar data suggest that degassing went on more rapidly earlier in the Earth's history than now, though uncertainties (such as the supply of potassium in the crust) make it difficult to attach a figure to this difference⁶. On our present argument, a more rapid early degassing would imply a lower average surface temperature. But, there remains one further parameter in equation (1) that requires examination—the flux factor, f . As with the albedo, the choice of a value for the flux factor in atmospheric models has been considered straightforward, and for the present-day Earth, there is no ambiguity over the flux factor to be used: $\tau_h/\tau_r \approx 10^2$, and $f = 4$. But the rotational period of the Earth is believed to have varied with time, owing to tidal interaction with the Moon, increasing from an original value of somewhat more than 2 h to the current figure of 24 h. If the Earth's atmosphere had undergone no major change with time, this rotational variation would clearly not affect the appropriate value of f . If, however, the terrestrial atmosphere has built up by degassing during the Earth's existence, then the value of f throughout the early period requires re-examination. It is generally supposed that the Moon was either captured, or formed in orbit, not later than 4,000 Myr ago. (Later capture should have led to observable effects in the geological record.) This implies that the major tidal interaction between the Earth and the Moon (and, therefore, the most rapid deceleration of the terrestrial rotation) occurred at the same time as our postulated initial degassing. Hence, both τ_h and τ_r would be changing simultaneously.

We have recomputed our values for the surface temperature of the early Earth taking into account concurrent changes in the value of f . (The rate of change of the Earth's rotation has been taken from Marsden and Cameron⁷.) The results are shown in Fig. 2. The significant consequence is that the average surface temperature of the early Earth is appreciably enhanced. This implies that changes in the albedo and flux factor can be set against each other to produce a range of possible temperatures for the early Earth. More particularly, the effects of an enhanced early degassing rate can be cancelled by an appropriately varying flux factor. Figure 2 clearly suggests that the early Earth may even

Fig. 1 Increase in the surface temperature of the Earth with increase in solar luminosity: *a*, for varying albedo; *b*, for constant albedo.



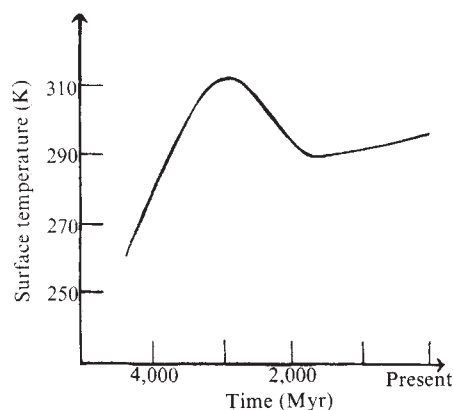


Fig. 2 Possible evolution of surface temperature for the Earth allowing for variations in the flux factor. These are caused by changes in terrestrial rotation due to the capture of the Moon.

have been warmer than the present-day Earth. It is, therefore, worth pointing out that some recent palaeotemperature measurements support this possibility⁸.

The model presented here is capable of fulfilling the boundary conditions without requiring the presence of additional absorbing agencies. More generally, we would argue that current atmospheric evolution models are too primitive to provide certain theoretical backing for an early reducing atmosphere on Earth. Hence, they must be used with great caution in discussions concerning the origin of life.

ANN HENDERSON-SELLERS
A. J. MEADOWS

Astronomy Department,
University of Leicester,
Leicester, UK

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Redox regulation of atmospheric oxygen and its consequences

MANY attempts¹⁻⁹ have been made to explain why the concentration of atmospheric oxygen remains steady at ~21%. I previously suggested that the turnover of marine and brackish water sediments promotes a recurrent demand for dissolved oxygen by alternately oxidising and reducing insoluble inorganic iron. Oxygen incorporated with sediments by this means combines with hydrogen to form water—thus preventing to some extent that loss of hydrogen by which Earth is oxidised^{4,7}. It is now apparent that regulation of molecular oxygen cannot be brought about by iron alone because the rate of turnover (even of shallow water sediments) is not fast enough¹³. Nor can sulphur (on its own or in association with iron) effect the transfer of sufficient oxygen from the aerobic to the anaerobic environment if rates of microbial activity in deep sea sediments are generally 1–2 orders of magnitude slower than in those of the continental shelves^{12,15}. I propose, therefore, that six elements (N, Mn, Fe, S, C, H) circulate in reduced and oxidised forms between the aerobic and anaerobic environments and bring about 'redox regulation of atmospheric oxygen'.

Entering the anaerobic environment as the oxidants NO_3^- , MnO_2 , FeOOH , SO_4^{2-} , CO_2 and OH^- , N, Mn, Fe, S, C and H

are reduced to N_2 , Mn(II) , Fe(II) , H_2S , CH_4 and H_2 at successively lower levels of redox potential—the reductions taking place in terrestrial soils, marine and freshwater sediments and the guts of animals. To complete their cycles (satisfying their oxygen demands as reductants) N_2 , Mn(II) , Fe(II) , H_2S , CH_4 and H_2 must be freed from the anaerobic environment. This happens by venting: $\text{N}_2\uparrow$, $\text{CH}_4\uparrow$ and $\text{H}_2\uparrow$, by diffusion: H_2S , and by turnover: Mn(II) and Fe(II) . The inorganic oxygen demands of Mn(II) , Fe(II) and H_2S are then satisfied by dissolved oxygen, whilst those of N_2 , CH_4 and H_2 are satisfied by reactive forms of gaseous oxygen ($[\text{O}]$, O_2 , O_3). On this basis all of them make recurrent demands on molecular oxygen though the demands of N, S and C (which circulate in vast quantities, combine with more than their own weights of oxygen and have their reductions brought about by microorganisms using dead organic matter as the reductant) have the greatest effect.

Redox cycling poises the aerobic environment at $pE \sim 13$ on an anaerobic environment extending from a level at which NO_3^- is reduced to gaseous nitrogen ($pE \sim 12$) to that at which water yields molecular hydrogen ($pE \sim -7$). ($pE (RTF^{-1} \ln 10) = Eh$ where Eh is the electrode potential of a half-cell measured against the standard hydrogen half-cell which by convention has a potential of 0V). In terms of pE , it is significant that regulation was achieved shortly after N became an oxygen sink, and that it is currently held at a level below that at which widespread forest and grassland fires would be limiting⁴. It is suggested that the 'gate' between the aerobic and anaerobic environments is 'guarded' by

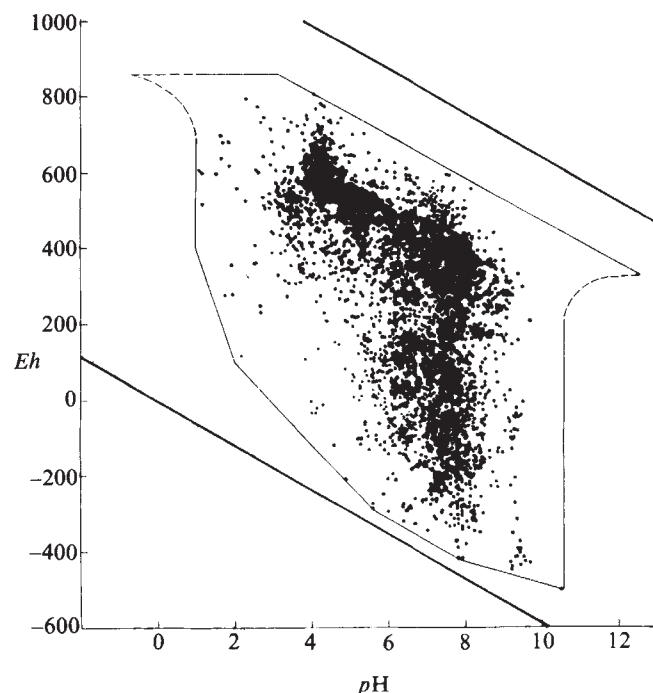


Fig. 1 Distribution of Eh - pH measurements of natural aqueous environments (after Baas Becking *et al.*¹⁰).

the processes of nitrogen fixation and denitrification. The former, using more molecules of oxygen than molecules of nitrogen, tends to lower oxidation potential to a point where the way is opened for denitrification. Such a lowering of pE can be viewed variously: as a displacement of one redox reaction by the one beneath it in the series, as a general rise of these reactions within a soil or sediment profile, as a slight telescoping of the anaerobic environment at the aerobic end. If nitrifying bacteria at the surface of a sediment or soil are inactivated by a lowering of oxidation potential, denitrifiers alongside them are activated and use the NO_3^- made elsewhere. Thus, normally, and in concert with the other cycles, the highly pE -sensitive N cycle is able to correct any tendency to over-regulation, so that despite the favourable pE and

pH of Earth's surface, an excess of NO_3^- (refs 5, 7, 11, 14) is as unlikely as a shortage of oxygen.

Functioning as a sink for oxidants, the reducing anaerobic environment helps to regulate the pH of the aerobic. This is evident in Fig. 1—a plot of some 6,000 Eh–pH readings (including those of other workers) made by Baas Becking *et al.*—which shows a marked columnar disposition of points between pH 7–8 in the anaerobic, surmounted in the aerobic by a linear concentration having the same slope (-59mV per pH unit) as the equilibria between water and oxygen and water and hydrogen. It is apparent that the reducing, anaerobic environment maintains a state of near neutrality throughout its range whilst the aerobic environment becomes more acid as it becomes more oxidised.

In the aerobic environment, this tendency to acidity as oxidants go into solution is offset by carbonate and aluminosilicate⁷ buffering. There are many places, however, where buffers are lacking and increases in acidity happen either because of a seasonal hiatus in the uptake and eventual reduction of oxidants (as in Norway where they are retained through winter in the snow) or because the anaerobic environment is too poorly developed to cope with the influx.

There are other places where the anaerobic environment is atypical and there are anoxic basins, in which oxidants are in short supply, and deserts, from which water and the reductants furnished by dead organic matter are missing. Earth is adjusted to their presence and it may be wrong to suppose that they are signs of environmental ill health. Nevertheless, overall, Earth's aerobic and anaerobic environments are mutually sustaining; to diminish one is to diminish the other; to have failed to acquire one would have meant failing to acquire both.

P. A. BOARD

24 Cottingham Avenue,
Horsham,
Sussex, UK

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organic matter for microbial consumption, and (4) contain oxidised nitrogen in the form of nitrate or nitrite. (The latter has been the only factor considered in most discussions of this problem¹.) According to Johnston², the fraction of denitrified nitrogen released to the atmosphere as N_2O is favoured by: (1) low temperature, (2) low pH, and (3) marginal anaerobic conditions; all of which in turn lead to a low rate of denitrification. Thus the net effect of these last three parameters on N_2O production was left ambiguous. Most authors seem to feel they cause a net reduction in the rate of N_2O production³.

Since taking up agriculture, man's tillage of the soil and elimination of natural standing water have had large scale effects.

First, soil oxygen has been increased through: (1) loosening and turning, producing increased aeration and (2) reduced competitive oxygen demand from root growth by: spacing of monocultured plants; elimination of competing plants or weeds, and fallowing; (3) reduction of oxygen-excluding soil moisture through: increased runoff; erosion and oxidation of water-retaining humus and non-return of plant debris; measures to improve drainage and/or percolation wherever water stagnation restricted plant growth.

Second, the volume of culture medium for soil bacteria has been reduced through the loss of humus and top soil.

There have, of course, been opposing effects favouring increased N_2O production from irrigation, rice culture and addition of fixed nitrogen to the soil but these are generally on a smaller scale, and tend to occur under conditions or in areas where they have little or no effect. For example, irrigation and to a lesser extent fertilisation is restricted to porous and well drained soils, large scale fertilisation has turned from nitrate to ammonia (oxidation of ammonia to nitrate will occur but only under conditions at least temporarily unfavourable for denitrification) and rice culture is restricted to areas already tending to be waterlogged. There have been numerous claims that anthropogenically produced oxides of sulphur and nitrogen have caused large scale increases in the acidity of precipitation and a suggestion⁴ that this could lead to increased production of N_2O . This is a logical theoretical extrapolation but it has little support from available data. As noted above, by slowing denitrification a lower pH may actually reduce N_2O production. Furthermore, there are large natural injections into the atmosphere of fixed gaseous sulphur and nitrogen exceeding those due to man. Without the return to the soil by the atmosphere of these soluble plant nutrients we could expect large biological deserts even where there is adequate rainfall. The areas in which decreased pH has been claimed represent only a small portion of the globe and past data to establish the trend are very fragmentary.

It thus seems plausible that any affect that man has had or will have in the foreseeable future on denitrification is to decrease N_2O production and thus to increase the depth of our ultraviolet-screen of stratospheric ozone. Available data on tropospheric concentrations of N_2O are very confusing⁵ and of little help in resolving this question. The data suggest abrupt increases in N_2O concentration between 1967 and 1968 and again in recent years. The difference, however, in N_2O concentration indicated by the different sets of measurements raises questions about the accuracy of the measurements. The ozone measurements are more encouraging. They suggest, that total global ozone has been increasing since measurements were first made in the early 1920s and particularly since the significant expansion in the observational network began about the mid-1950s.

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HUGH W. ELLSAESSER

Lawrence Livermore Laboratory,
University of California,
Livermore, California 94550

Has man increased stratospheric ozone?

MANY anthropogenic threats to the ozone layer have surfaced (eight so far) since McDonald first suggested that supersonic transport aircraft would lead to increased skin cancer. It should be comforting, therefore, that agricultural modifications to the Earth (increased oxygenation, drainage, and pH of the soil and destruction of bacteria culturing humus) are suggested here to be more important than addition of fixed nitrogen in modifying N_2O production by denitrifying bacteria. If true, man should have caused an increase in total global ozone.

This effect operates through the denitrification branch of the nitrogen cycle in which soil bacteria, in the absence of oxygen, turn to nitrate and nitrite as oxygen donors and produce N_2 and N_2O as waste byproducts which escape into the atmosphere. Part of the N_2O ascends by the Hadley circulation into the stratosphere where a fraction, estimated at about 1% of the total surface production, is oxidised to produce the catalytic destroyer of ozone—NO. While most of this cycle is known only qualitatively, it is believed that denitrification is favoured in soils which: (1) are saturated with water, (2) are largely devoid of oxygen, (3) contain

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Evidence of rapid changes in the Permian geomagnetic field during the Zechstein marine transgression

CONSIDERABLE refinements have been made recently to Palaeozoic magnetic reversal stratigraphy. Here, we present evidence of a previously unrecognised rapid field change of positive polarity during the Upper part of the Kiaman Magnetic Interval. This is recorded in the Upper Permian Marl Slate, a well-known stratigraphic marker horizon and should be of considerable value to future palaeomagnetic and stratigraphic studies.

Late Palaeozoic palaeomagnetic data are voluminous, largely because of the ubiquity of easily measured red beds and igneous rocks. Within these data there are systematic discrepancies and it has been suggested that the Permian geomagnetic field did not perfectly correspond to an axial dipole. Palaeontological diversity data have also been used to argue that the Permian field was non-dipole¹. A number of palaeomagnetists have noted that pole positions plotted with respect to the Bullard *et al.*² reconstruction are, for the southern continents, displaced by about 20° to the east, although this has been explained in terms of an alternative reconstruction³. Briden *et al.*⁴ investigated Permian and Triassic palaeomagnetic data in detail and were able to show that, at a first approximation, the Permian field corresponds to an axial dipole when based on a Bullard-type fit. One of the important discrepancies in this model is that for European data, inclinations are often much less than expected. These were interpreted in terms of coaxial multipole fields and our results provide further evidence of short lived anomalies in the Permian geomagnetic field. We still believe, however, that the best overall model of the Permian field is that of a geocentric axial dipole.

An important feature of the Permian geomagnetic field is that it was persistently of reversed polarity. From late Carboniferous to late Permian times, the geomagnetic field was predominantly reversed and this has been called the Kiaman Magnetic Interval. One brief normal event occurring at approximately the Carboniferous–Permian boundary has been documented in the USSR and the USA⁵. Here we record the discovery of further rapid field changes of positive polarity occurring at a stratigraphically significant horizon which could be of considerable value to studies of Permian strata.

Our results come from an investigation of the role of metal sulphides in palaeomagnetism and, specifically, from the Upper Permian Marl Slate. This is a thin, black, sapropelic, dolomitic laminite which represents the Zechstein marine transgression over Rotliegendes sandstones in northwestern Europe⁶. The eastern European equivalent of the Marl Slate is the German Kupferschiefer. A complete sequence of the Marl Slate has been obtained from a borehole drilled off the north-east coast of England by the National Coal Board at NZ5099852007 and donated for study by Dr D. Magraw. In this borehole, the Marl Slate was cored from a depth of 285.3 m and is 1.18 m thick. Thirty-six cores of 2.5-cm diameter were drilled parallel to the bedding and throughout the length of the core. In this way, palaeomagnetic directions could easily be referred to the bedding planes and true palaeolatitudes calculated. Only the relative declination could be measured, because the absolute orientation of the core relative to geographic North is unknown. The natural remanent magnetisation (NRM) of the specimens was measured along with their low-field susceptibility. The results are summarised in Fig. 1. The NRM intensity of the Marl Slate is weak, of the order of 0.1 μG and the initial susceptibility varies between 5 and

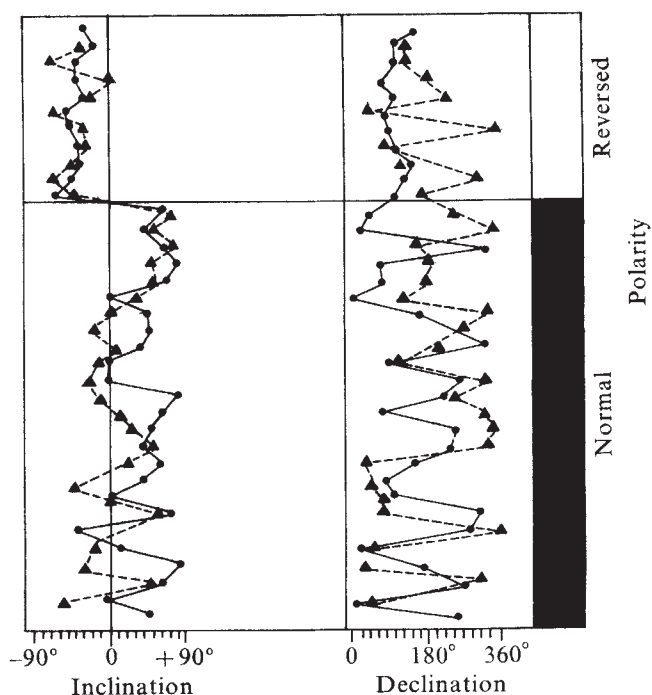


Fig. 1 Magnetic stratigraphy of the Marl Slate showing an upper zone of negative polarity and a lower zone of positive polarity. The section is 1.18 m thick. Declination is relative, inclination is absolute. The results are shown before heating (●) and after heating (▲).

14 $\mu\text{G Oe}^{-1}$. The initial directions fall into two well defined groups: an upper zone with negative inclinations, and a lower zone of positive inclinations. The mean direction of magnetisation is dec. 299°, inc. +34°, α_{95} 19°, after the polarity of the upper group has been normalised. In these initial measurements, there are important differences in the degree of scatter of both inclination and declination between the positive and negative zones. In the upper, negative part of the core, both declination and inclination show much less systematic variation than the lower, positive part of the core (Fig. 1). The transition from positive to negative inclination is extremely sharp but there is no sharp transition in declination, although this may be due to the fact that the directions have relatively steep inclination, and would not be expected to show such a sharp transition.

Thermal demagnetisation studies investigated the stability of the magnetisation and showed that the magnetisation of the Marl Slate in this core is extremely soft and only about 20% of the original remanence remains after heating to 200 °C. At and above this temperature, there are large increases in intensity due to a laboratory magnetisation caused by the oxidation of sulphide minerals⁷. During thermal demagnetisation, no recognisable secondary components were removed and after cleaning the specimens at 150 °C, the positive and negative zones in the core remained unaffected. There was little change in the mean direction of magnetisation of the core: dec. 324°, inc. +21°, α_{95} 34°, although there was some increase in scatter, particularly in the declination. This results from the difficulty of resolving accurately the magnetisation of the specimens at much weaker NRM intensities (average 0.05 μG). For these reasons, we believe the initial measurements represent most accurately the original magnetisation of the Marl Slate.

Several factors suggest that the magnetisation of the Marl Slate in this core was acquired during deposition or early diagenesis. Because the core came from a submarine borehole, the specimens should be free from surface oxidation and this has been confirmed by detailed mineralogical investigations.

The presence of iron, zinc, copper and lead sulphides, of early diagenetic origin and showing no signs of subsequent alteration is taken as evidence that the original magnetic mineralogy is largely unchanged. Moreover, textural evidence indicates that the main mineral phase in the Marl Slate, dolomite, is also of early diagenetic origin. It is interlaminated with calcite and consists of thin laminae of dolomicrite and hypidiotopic dolomite⁸, features which indicate an early diagenetic origin. The NRM carrier cannot as yet be identified precisely, although rock magnetic studies indicate that it might be a mixture of magnetite and haematite.

X-ray diffraction and geochemical studies indicate that the lower part of the core is richer in detrital quartz, whereas the upper part has a higher carbonate content. We take this to indicate that the sedimentation rate was relatively rapid in the lower part and much slower in the upper part of the core (corresponding approximately to the negatively magnetised zone).

We are therefore able to interpret the magnetisation of the Marl Slate. The upper part represents a negatively magnetised zone, acquired over a relatively long time and incorporating ambient geomagnetic field changes. The lower part is a zone of positive magnetisation, acquired more rapidly and in which ambient geomagnetic fluctuations have not been averaged out. Although the upper negative zone may represent a part of the Kiaman reversed polarity, we do not believe that the lower positive zone is a simple normal polarity zone, but rather evidence of rapid field changes similar to an excursion. We believe that the recognition of this positive rapid field change in the upper Kiaman is particularly significant because of its stratigraphic location. It coincides with strata which record the Zechstein marine transgression and which can be widely recognised over northwestern Europe. Further palaeomagnetic studies should provide a better understanding of the timing of this transgression and also of the Permian geomagnetic field.

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P. TURNER
D. J. VAUGHAN

Department of Geological Sciences,
The University of Aston in Birmingham,
Birmingham, UK

Received 8 September; accepted 4 November 1977.

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Hilgardite and parahilgardite piezoelectric zeolite type phases

THE calcium chloroborate minerals hilgardite and parahilgardite are classic examples of point group symmetries *m* and *l* respectively^{1,2}. They were found in the Choctow salt dome, Louisiana and are both piezoelectric. The chemical composition, originally given as $\text{Ca}_8(\text{B}_5\text{O}_{11})_4\text{Cl}_4 \cdot 4\text{H}_2\text{O}$ for both minerals (see refs 1 and 2), has been revised to $\text{Ca}_2(\text{B}_5\text{O}_8(\text{OH})_2\text{Cl})$ (ref. 3). Our structure determination indicates the chemical composition to be $\text{Ca}_2\text{B}_5\text{O}_9\text{Cl} \cdot \text{H}_2\text{O}$ for both phases.

Hilgardite is monoclinic, space group *Aa*, with cell dimensions: $a = 11.438(2)$, $b = 11.318(2)$, $c = 6.318(1)$ Å, $\beta =$

$90.06(1)^\circ$; $Z = 4$. Parahilgardite is triclinic, space group *P1* with cell dimensions: $a = 17.495(4)$, $b = 6.487(1)$, $c = 6.313(1)$ Å, $\alpha = 60.77(1)$, $\beta = 79.56(1)$, $\gamma = 83.96(2)^\circ$; $Z = 3$. The crystal structures have been determined by the heavy atom method and refined by the method of least squares to *R*-factors of 0.018 and 0.028 for hilgardite and parahilgardite respectively, based on 1,475 and 4,432 reflections, measured on an automatic single crystal diffractometer.

The structure of hilgardite is a three-dimensional borate framework, whose building block is the anhydrous pentaborate polyanion, $(\text{B}_5\text{O}_{12})^{3-}$, consisting of three (BO_4) -tetrahedra and two (BO_3) -triangles. Because all of the terminal oxygen corners are shared, the chemical composition of the borate framework is $(\text{B}_5\text{O}_9)^{3-}$. The pentaborate polyanions form chains parallel to the *c*-axis by sharing tetrahedral corners with those belonging to adjacent polyanions (Fig. 1). Within each chain, corners of two borate tetrahedra are pointed along $+a$ and $+b$ directions, whereas corners of two borate triangles are pointed along $-a$ and $-b$ directions; these corners are shared with four adjacent chains (Fig. 2), such that tetrahedral corners of one chain are shared with triangular corners of the other. An open borate framework is created this way, which has channels (diameter ~ 5.2 Å) parallel to the *a*- and *c*-axes. The water molecules and the chlorine atoms occur within the channels,

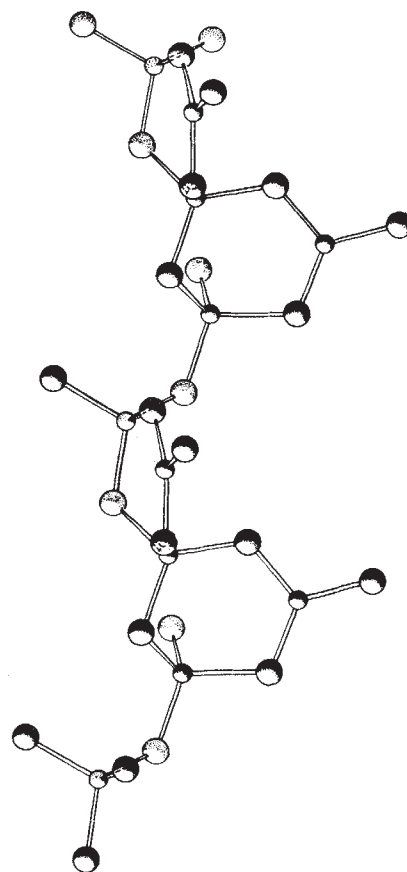


Fig. 1 The pentaborate polyanion chain in hilgardite parallel to the *c*-axis. A similar chain exists in parahilgardite.

whereas the calcium atoms occur at the edge of the channels running parallel to the *c*-axis. The crystal structure of parahilgardite is very similar to that of hilgardite, consisting of an open borate framework, formed of three crystallographically independent pentaborate units, where the calcium and chlorine atoms occur within open channels. Thus, hilgardite and parahilgardite are zeolite type phases. To our knowledge, this is the first report of borate zeolites. Ion-exchange experiments on these two minerals are in progress.

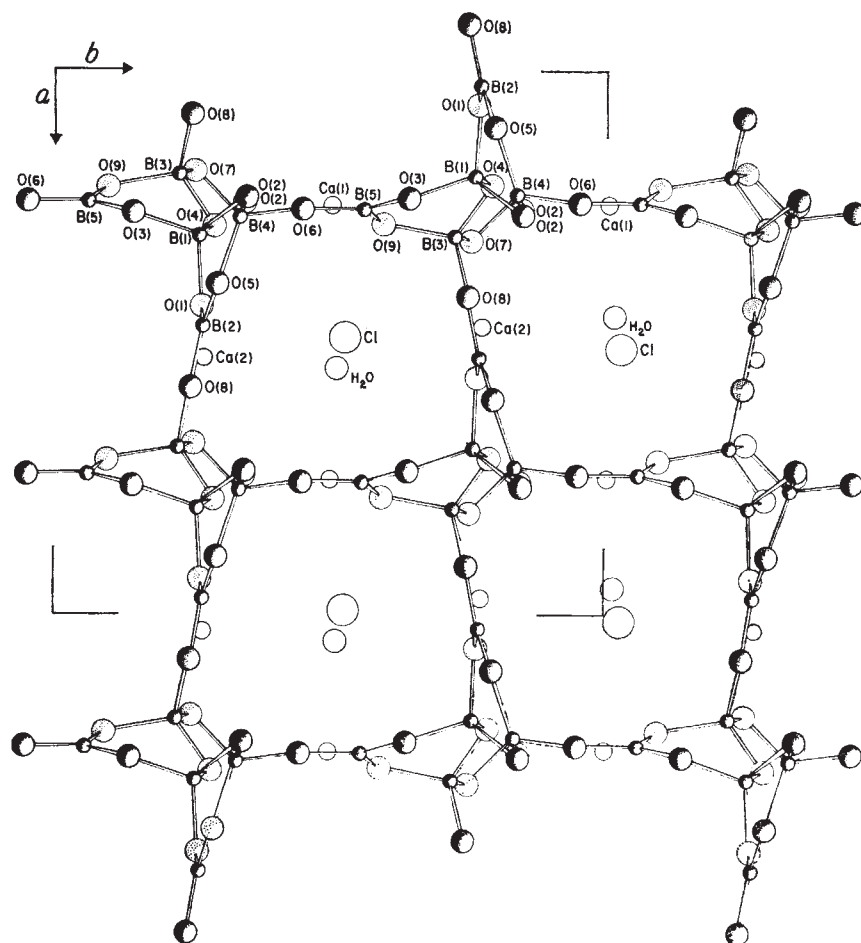


Fig. 2 The pentaborate framework structure of hilgardite projected down the *c*-axis, showing open channels (diameter 5.2 Å) parallel to *c*. Note that the chlorine atoms and water molecules occur within these channels.

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SUBRATA GHOSE
CHE'NG WAN

Department of Geological Sciences,
University of Washington,
Seattle, Washington 98195

Received 20 July; accepted 5 August 1977.

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Responses of a benthic marine invertebrate to γ -irradiated sediment

THERE seems to be little information on the influence of high radiation levels on marine sediments and their subsequent recolonisation by sedimentary invertebrates. We report here that the benthic amphipod *Corophium volutator* (Pallas) responds to irradiated sediments in which there have been changes of *Eh* and microbial viability. Our results are of direct relevance to the microbiological and chemical properties of sediments¹⁻³, and to the role played by microorganisms in habitat selection by benthic invertebrates^{4,5}.

The first series of experiments (Table 1) demonstrates behavioural responses of *C. volutator* to irradiated compared with autoclaved and untreated sediments. Mud sediments were collected intertidally from the Clyde Estuary and wet-sieved through a 750- μ m sieve to remove large particles. Batches of sediment were

then divided into three parts. One part was the untreated control. The second was autoclaved at 121°C for 30 min and the third was exposed to γ irradiation for 18 h, giving a dose of 6 Mrad (cobalt source, Scottish Research and Reactor Centre, East Kilbride). Precautions were taken to ensure even irradiation and samples were immersed in a large water bath to prevent heating effects during irradiation.

Behavioural experiments were conducted with *C. volutator* in which animals were offered a choice of two sediments using standard techniques⁴. Animals preferred control to irradiated sediment and control to autoclaved sediment (Table 1). When autoclaved sediment was tested against irradiated, however, animals markedly preferred the autoclaved. This result was unexpected and led us to measure the physical and chemical properties of the autoclaved and irradiated sediments, to establish any differences between them. The most clearly defined difference was in redox potential. On replicate measurements the redox potential of the autoclaved sediment was -110 to -130 mV and of the irradiated sediment was -550 to -590 mV. Control sediments had an *Eh* of -50 to -65 mV.

We then investigated the relationship between increasing irradiation and *Eh*; heterotrophic bacterial counts were also taken (surface counts on Difco marine agar). The result (Fig. 1) shows that the heterotrophic bacteria decreased from 2×10^7 cells per g dry weight in the unirradiated sediment to less than 1×10^2 cells per g dry weight in the sediments irradiated with 0.5 Mrad or more. On the other hand *Eh* remained constant to 1.5 Mrad, and then declined to very low values of -500 to -600 mV at 4 to 6 Mrad. The difference in the shape of the two curves allowed us to select irradiation treatments of 0.8 Mrad and 5.4 Mrad which would give sediments of normal *Eh* (about -100 mV) and very low *Eh* (about -600 mV), respectively, while both would be virtually sterile (less than 10 cells per g). These two irradiation

Table 1 Preferences of *C. volutator* for control, irradiated and autoclaved sediments

Expt	control sediment	% animals in autoclaved sediment	Total no. animals burrowed
(1)	66	34	85
Expt	control sediment	% animals in irradiated sediment	Total no. animals burrowed
(2)	78	22	87
Expt	autoclaved sediment	% animals in irradiated sediment	Total no. animals burrowed
(3)	71	29	45

Each experiment was run in triplicate using three choice dishes⁴. Between 28 and 35 animals were added to each dish. Experiments were run for 1 h. In each experiment, χ^2 was applied to the numbers of preferring the two choices in the three dishes, in a 2×3 contingency test. The χ^2 values for all three experiments were not significant, showing that within each experiment the results were homogeneous. The result from the three dishes in each experiment were therefore summed to give the data in the table. χ^2 was then applied to the numbers burrowed in the two choices in each experiment, and was significant: experiment 1, $\chi^2 = 8.58$, 1 d.f. $0.005 > P > 0.001$; experiment 2, $\chi^2 = 27.60$, 1 d.f. $P < 0.001$; experiment 3, $\chi^2 = 8.02$, 1 d.f. $0.005 > P > 0.001$. The number of animals not burrowed at the end of the experiment was small in experiment 1 and 2 (10 and 4% respectively), but larger in experiment 3 (35%) which is accounted for by the relative unsuitability of both autoclaved and irradiated sediments as assessed by experiments 1 and 2.

levels, which are marked with broad arrows in Fig. 1, were chosen for further experiments.

The behavioural response of *C. volutator* to 0.8-Mrad, 5.4-Mrad and control sediments was assessed (Table 2). *Corophium* could not distinguish between the control and the 0.8-Mrad sediments, but clearly preferred the 0.8-Mrad to the 5.4-Mrad sediment, and the control to the 5.4-Mrad sediment. The lack of difference between the control and 0.8-Mrad sediments shows that the absence of viable bacteria does not reduce the attractiveness of the sediments: in other words, *C. volutator* cannot distinguish between living and dead microorganisms. The marked avoidance by animals of the 5.4-Mrad sediments when compared with the 0.8-Mrad sediment or the control proves that animals avoid very low *Eh* values or some other associated change that occurs at high irradiation levels.

We believe that our results are relevant to the role played by microorganisms in habitat selection by benthic invertebrates⁵⁻⁹.

Table 2 Preferences of *C. volutator* for control, 0.8-Mrad and 5.4-Mrad sediments

Expt	control sediment	% animals in 0.8-Mrad sediment	Total no. animals burrowed
(1)	53	47	93
Expt	control sediment	% animals in 5.4-Mrad sediment	Total no. animals burrowed
(2)	92	8	99
Expt	0.8-Mrad sediment	% animals in 5.4-Mrad sediment	Total no. animals burrowed
(3)	81	19	97

Each experiment was run in triplicate using three dishes⁴. Between 30 and 40 animals were added to each dish. Experiments were run for 1 h. χ^2 showed that the triplicate results were homogeneous and so the results for each experiment were summed to give the data in Table 2 (see legend to Table 1 for statistical details). χ^2 applied to the number of animals burrowing in the two choices in each experiment was not significant in experiment 1 ($\chi^2 = 0.27$, 1 d.f. $0.7 > P > 0.5$) but highly significant in experiments 2 and 3 (experiment 2, $\chi^2 = 69.59$, 1 d.f. $P < 0.001$; experiment 3, $\chi^2 = 38.36$, 1 d.f. $P < 0.001$). In all three experiments very few animals had not burrowed by the end of the experiment (0-3%).

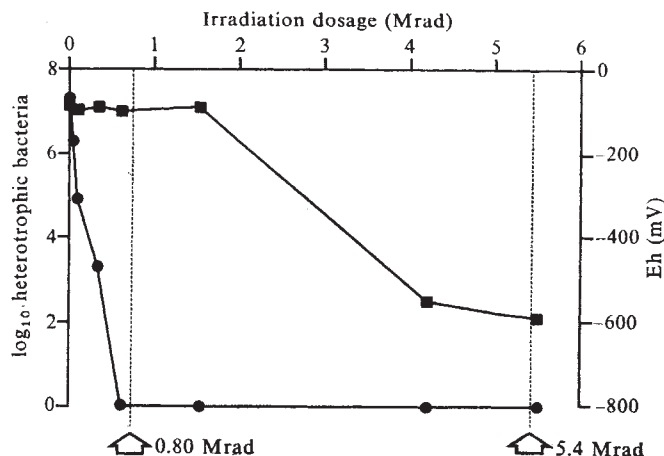


Fig. 1 Sediment heterotrophic bacteria (●) and redox potential (■) in relation to irradiation dosage. The two broad arrows relate to the irradiation levels used in Table 2 (see text).

Most authors have stressed the importance of microorganisms as stimulus to sediment recognition, but their deductions have been based on the use of drastic physical and chemical treatments which alter the overall physico-chemical characteristics of sediments⁵. The lower of the two levels of irradiation used by us (0.8 Mrad, Fig. 1) does not seem to alter these characteristics but kills sediment microorganisms. Our findings show that in these circumstances *C. volutator* does not distinguish between living and dead cells (Table 2). We therefore conclude that the viability of sediment microorganisms may be of little significance in habitat selection and that their role lies in their effect on the chemical and physical properties of the sediment.

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E. A. DEANS
J. G. ANDERSON
P. S. MEADOWS

Department of Applied Microbiology,
University of Strathclyde
and Department of Zoology,
University of Glasgow, UK

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Diversity of bacterial populations in the Beaufort Sea

BIOLOGICAL communities usually contain few species with many individuals and many species with few individuals¹. Diversity of higher organisms generally has been found to decrease with increasing population size¹. Communities in physically stressed ecosystems, such as in polar regions, characteristically have low species diversity². Diversity indices have been used to express species diversity in plant and animal communities, especially as a measure of stress on community structure². Amongst microorganisms, diversity indices have been applied to microscopic phytoplankton populations^{3,4}, but not to bacterial populations. Bacterial populations, however, show large variations in species

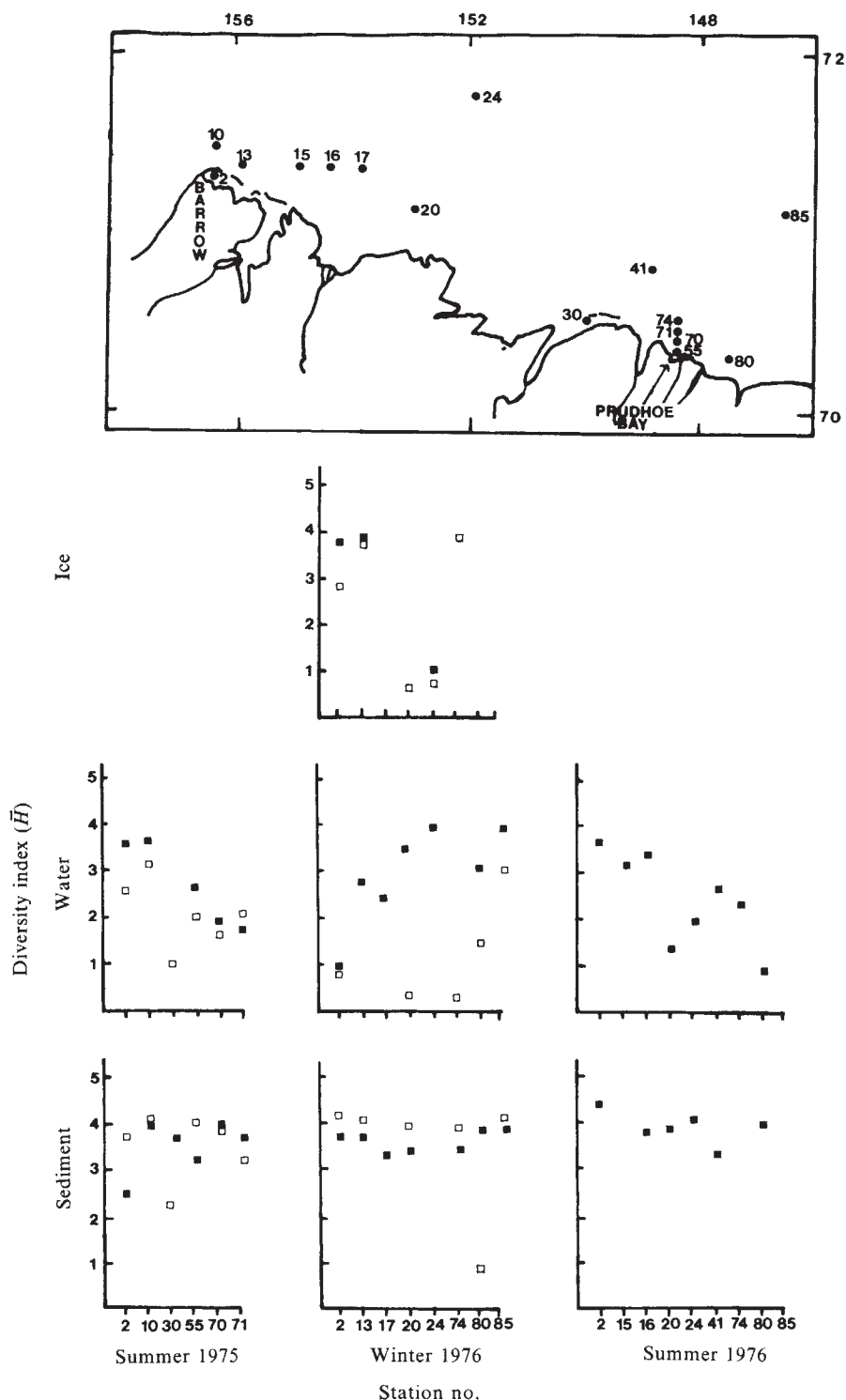


Fig. 1 Sampling sites in the Beaufort Sea and bacterial diversity indices during summer and winter in ice, water and sediment at these sites. Closed symbols, populations isolated at 4 °C, open symbols, populations isolated at 20 °C.

diversity. For example, infections are dominated by one or a few species, whereas many bacterial species are found in an ecosystem such as soil⁵. We report here the application of taxonomic diversity indices to bacterial populations and a study of the relationship of the diversity indices of such populations to population size and to population-independent factors such as geographic location.

It is difficult to define bacterial species and identify them in natural ecosystems. Unlike plants and animals, including even microscopic protozoa and algae, it is not possible to identify bacterial species by visual observation alone. We used numerical taxonomic procedures to define phenotypic clusters of bacteria, which we considered functionally equivalent to species. These clusters were used to calculate the species diversity of bacterial populations in nearshore areas of the Beaufort Sea.

Water and sediment samples were collected during late summer (August–September) 1975 and 1976 and late winter (March–April) 1976 at various sites in the western Beaufort Sea (Fig. 1). During winter, ice samples also were collected. Average surface water temperatures were about +1 °C in summer and –1.8 °C in winter. Samples were maintained at 4 °C during processing. Viable heterotrophic bacterial populations were enumerated by plating serial dilutions on marine agar 2216 (Difco). Duplicate series of plates were incubated at 4 °C for enumeration and isolation of psychrophilic–psychrotrophic populations, and at 20 °C for mesophilic populations. Details of these enumeration studies will be reported separately.

All of the bacterial colonies on the countable plates were isolated in pure culture. Bacterial isolates, 20–25 per sample, were selected at random for characterisation. Approximately 300

features were determined for each isolate, including morphological, physiological, biochemical and nutritional characteristics. The Jaccard coefficient (S_j) and single linkage cluster analyses were used to sort the bacteria according to phenotypic similarity⁶. Strains related at greater than 70% similarity were considered to constitute a taxonomic grouping. Known strains of bacteria, included as controls, were separated into species at this level using the same testing procedures. The number of taxonomic groups and the number of individuals in each group, determined by the cluster analyses, were used to calculate the Shannon diversity index, $\bar{H}$ (refs 7–10). The formula $\bar{H} = c/N(N \log_{10} N - \sum n_i \log_{10} n_i)$ was used, where $c = 3.3219$, N = total numbers of individuals and n_i = total number of individuals in the i th taxonomic grouping.

The diversity of the psychrophilic–psychrotrophic and mesophilic populations generally showed similar patterns of geographic variation (Fig. 1). Almost all of the bacterial strains were psychrotrophic or psychrophilic, as is found in most cold marine water and sediment samples¹¹. Moving eastwards from Barrow towards Prudhoe Bay and northwards from Prudhoe Bay, bacterial diversity in water decreased during both summers, but increased during winter. No regular pattern of diversity between sampling sites was found in sediment or ice samples. Population levels in water were higher during summer 1975 and lower during winter 1976 near Prudhoe Bay than near Barrow. During summer 1976, populations were uniformly very high in all water samples.

Bacterial diversity in surface waters was inversely related to population size only at population levels below 10^4 ml^{-1} (Fig. 2). During summer 1976 when bacterial populations in water were above 10^4 ml^{-1} , no correlation between population size and

diversity was found. During summer, 1975, populations in water above 10^4 ml^{-1} generally had diversities of less than 2; populations less than 10^4 ml^{-1} had diversities of between 2 and 4 that were inversely related to population size.

During winter when bacterial populations in water were generally below 10^4 ml^{-1} , bacterial diversity clearly increased with decreasing population size. At the lower population levels generally found in water in winter, bacterial diversity was high. But, when population levels were similar in water samples collected in summer and winter, diversity was always lower in the winter samples. We consider the observed seasonal temperature variation of about 2°C insufficient to explain the observed differences in population size or diversity. Nutritional studies showed that these populations had extensive growth factor requirements. Presumably, these nutritional requirements are supplied by primary productivity of phytoplankton. Lack of light reaching the water during winter, thus may be a severe physical stress on the bacterial community, perhaps indirectly through phytoplankton productivity.

Compared with summer samples, a lower percentage of the strains isolated at 4°C from winter water samples, was capable of growth at 20°C . Selective pressure on mesophiles may account for differences in diversity between populations isolated at 4 and 20°C during winter.

In sediment, bacterial populations were generally greater than 10^4 g^{-1} , regardless of sampling time. $\bar{H}$ for bacterial populations in sediment was approximately 4, regardless of population size or sampling time. No correlation between population size and diversity was found for bacterial populations in sediment. $\bar{H}$ for

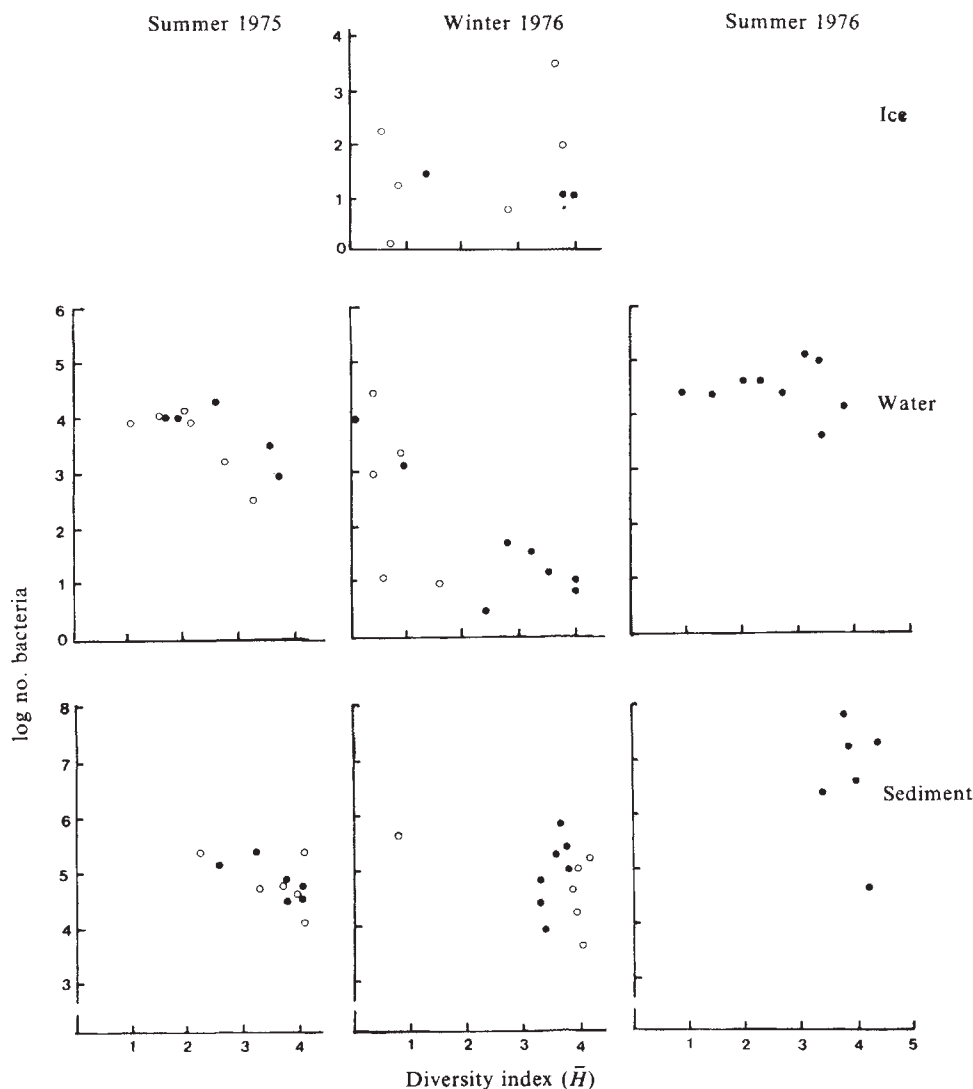


Fig. 2 Relation of population size to bacterial diversity in ice, water and sediment. Closed symbols, populations isolated at 4°C , open symbols, populations isolated at 20°C .

populations in sediment was generally higher than in water. Sediment populations are probably not subjected to the seasonal physical stress from temperature, salinity and light intensity changes as are water populations.

Although population levels were less than 10^4 ml⁻¹ in sea ice samples, no correlation between population and diversity index was found for populations from ice. This probably reflects the fact that bacteria are frozen into the ice matrix and survive but do not grow. Therefore, selection pressures are not operative in ice.

In summary, numerical taxonomic testing of randomly selected bacterial strains can be readily used to calculate bacterial diversity. Bacterial diversity in Beaufort Sea water showed reciprocal geographic trends between summer and winter. While bacterial populations in water were generally lower during winter, as predicted, probably due to the physical stress, diversity at most sites remained as high as during summer. Bacterial populations up to a threshold population size showed an inverse relation between diversity and population size, as occurs in populations of higher organisms. Beyond the threshold size, the diversity index did not correlate with population size. Even above the threshold population size, though, the diversity index showed geographic differences in community structure for bacterial populations in water.

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TATSUO KANEKO
RONALD M. ATLAS

Department of Biology,
University of Louisville,
Louisville, Kentucky 40208

MICAH KRICHEVSKY

Microbial Systematics Unit,
NIDR-NIH,
Bethesda, Maryland 20014

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Dosage compensation for an X-linked gene in pre-implantation mouse embryos

THERE is strong evidence that one of the two X chromosomes is inactive in somatic cells of adult female mammals, so that the effective gene dosage per cell for X-linked loci is the same in XX females as in XY males or XO females. This system of dosage compensation is thought to be established soon after implantation¹—certainly both X chromosomes seem to be potentially active in the blastocyst². To test for dosage compensation before implantation one can study the activity of an X-chromosome-coded enzyme that is synthesised during pre-implantation development, as a result of *de novo* transcription, in male and female embryos. Two such enzymes are considered to be hypoxanthine phosphoribosyl transferase (HGPRT EC 2.4.2.8) and α -galactosidase^{3,4}. Epstein³ found that groups of unfertilised

eggs from XX mothers had twice the HGPRT activity found in those from XO mothers, indicating that both X chromosomes are functional during oogenesis^{3,5}. By the morula and blastocyst stage, HGPRT activities had increased strikingly and the twofold difference had disappeared, implying that the enzyme present was no longer maternal in origin. Because pooled male and female embryos were assayed it was impossible to determine whether dosage compensation was occurring. Adler *et al.*⁴ measured α -galactosidase activity in single pre-implantation embryos, expecting that the presence of both female and male embryos would give rise to either a bimodal or a unimodal distribution of activities in the absence, or presence, respectively, of dosage compensation. The assay was insufficiently precise to give unequivocal results. We have now developed a single-embryo assay for HGPRT of sufficient precision to establish that the distribution of activities in single embryos, half of which we expect to be XX and half XY, is unimodal at both the eight-cell and the blastocyst stage. We conclude that dosage compensation occurs during pre-implantation development.

For single-embryo assays, where variation in the recovery of cytoplasmic contents would be expected to contribute substantially to the variance of the activity measurements, increased accuracy is achieved by the simultaneous measurement of activities of HGPRT and of an autosomal enzyme (adenine phospho-ribosyl transferase, EC 2.4.2.7) in the same reaction mixture. The results are expressed as a ratio of HGPRT:APRT activities in the individual

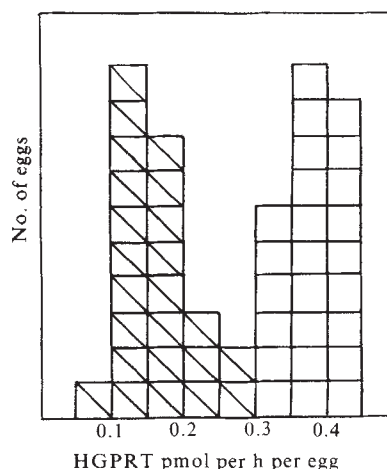


Fig. 1 Assays of HGPRT were carried out on batches of five to 10 unfertilised eggs from XX and XO (hatched squares) females (genotypes Ta/+ and +/O). Superovulation was induced by intraperitoneal injection of 7.5 IU of pregnant mare serum and 48 h later, 7.5 IU of human chorionic gonadotrophin. Eggs were isolated from the oviducts on the next morning, cumulus cells were removed with hyaluronidase and the eggs were washed carefully several times. The media used for collection of eggs and embryos (Figs 2 and 3) and the methods of isolation are described in ref. 8. The batches of eggs were transferred in less than 5 μ l of PB1 medium into individual microcaps and extracts were prepared by freeze-thawing in liquid nitrogen three times, followed by centrifugation at 2,000 r.p.m. for 10 min. The supernatants were added to 50 μ l volumes of reaction mixture. Reaction mixtures contained (final concentration) 35 mM sodium phosphate buffer (pH 7.4), 5 mM magnesium chloride, 2 mM phosphoribosyl pyrophosphate, 10 μ M ³H-guanine (specific activity 0.084 Ci mmol⁻¹) and 10 μ M ¹⁴C-adenine (specific activity 58 mCi mmol⁻¹). The reactions were carried out at 37 °C for 3 h and stopped by addition of 1 ml of 0.1M ice-cold lanthanum chloride containing 1 mM adenine and 1 mM guanine. Extracts from 25 batches of XX eggs and 24 batches of XO eggs were assayed individually; the mean activities of HGPRT (pmol per egg per h $\pm$ s.e.) were 0.383 ± 0.001 and 0.164 ± 0.002 , respectively. The mean activities of APRT (pmol per egg per h $\pm$ s.e.) were 0.17 ± 0.01 and 0.21 ± 0.01 for XX and XO eggs, respectively (data not shown).

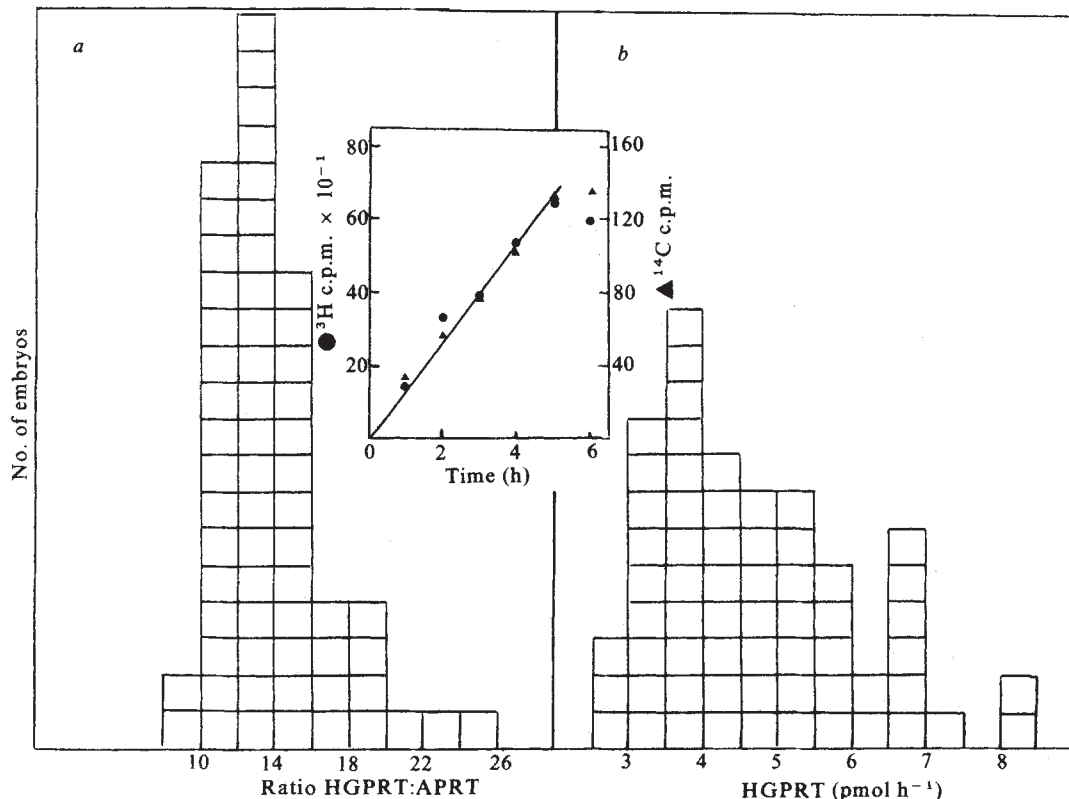


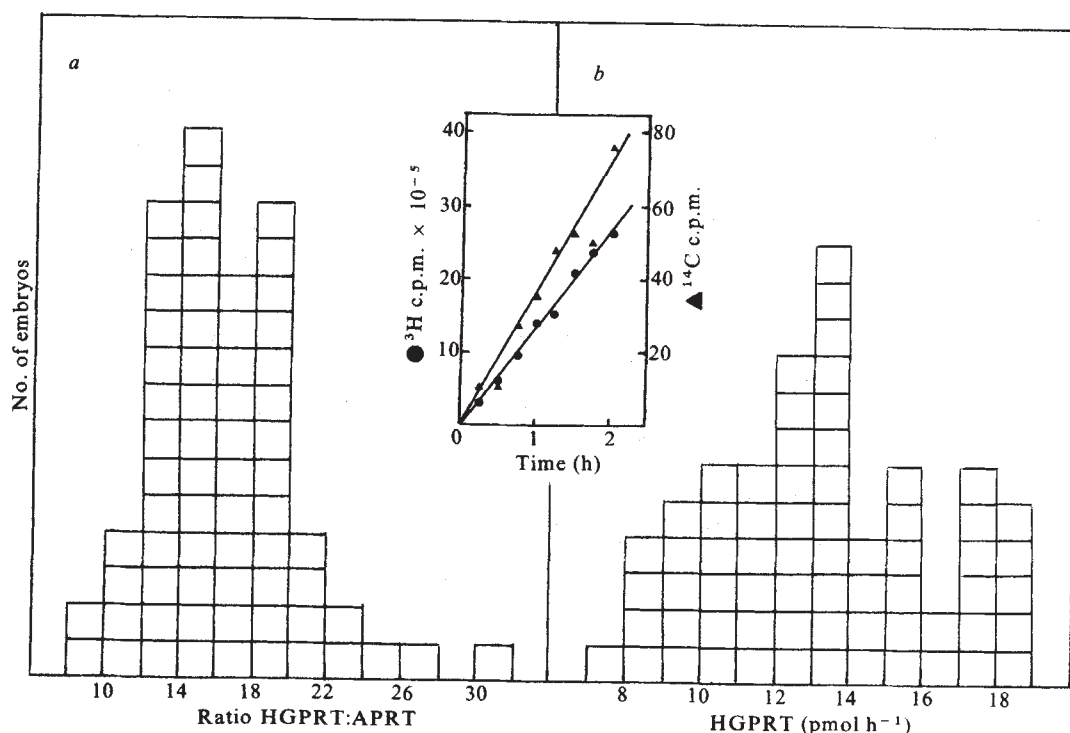
Fig. 2a, Ratio of activities of HGPRT to APRT in 62 single eight-cell embryos isolated from oviducts from MF1 females (Olac 1976 Ltd) on the third day of pregnancy. Each square represents the ratio obtained for a separate eight-cell embryo. Individual embryos were isolated into microcaps. The preparation of embryo extracts and the assay procedure are described in the legend to Fig. 1. Assays were carried out at 37 °C for 4 h. Data from two experiments are pooled. The mean HGPRT and APRT activities (pmol per h per embryo $\pm$ s.e.) were 4.697 ± 0.172 and 0.346 ± 0.013 , respectively. The inset figure shows both enzyme reactions to be linear in eight-cell embryos for at least 5 h. **b**, Activities of HGPRT in single eight-cell embryos.

embryo. The simultaneous assay of the two enzymes is based on the conversion by cell-free extracts of ³H-guanine and ¹⁴C-adenine to their respective monophosphates⁶. These are precipitated with lanthanum chloride and collected on filters, and the ratio of the two isotopes is determined.

To establish the validity of our assay system we first measured HGPRT activity in groups of unfertilised eggs from XX and XO females and confirmed Epstein's findings³

of an approximate twofold difference (Fig. 1), whereas the activity of APRT was nearly the same (see legend to Fig. 1). As further controls, we assayed extracts of cell lines lacking HGPRT (for example, an HGPRT-deficient derivative of PSA4⁷); these gave no incorporation of ³H-guanine into precipitable product, though high APRT levels were found. Also, the omission of the substrate, phosphoribosyl pyrophosphate (PRPP) from the single-embryo

Fig. 3a, Ratios of activities of HGPRT to APRT in 67 single blastocysts isolated from the uteri of MF1 females on the fourth day of pregnancy. Each square represents the ratio obtained for a separate blastocyst. Individual blastocysts were isolated into microcaps. The preparation of extracts and the assay procedure are described in the legend to Fig. 1. Assays were carried out at 37 °C for 2 h. The data from two separate experiments are pooled. The mean HGPRT and APRT activities (pmol per embryo per h $\pm$ s.e.) were 13.269 ± 0.367 and 0.822 ± 0.021 , respectively. The inset shows that both enzyme reactions are linear in blastocyst extracts for at least 2 h (the specific activity of the ³H-guanine in this timed experiment was 8.4 Ci mmol⁻¹ instead of 0.084 Ci mmol⁻¹). **b**, Activities of HGPRT in single blastocysts.



assays inhibited both enzymatic reactions. Such factors as breakdown of substrates, nucleotide pool sizes and enzymes which may destroy the nucleotide products, may well vary between stages of development, but would not be expected to differ between female and male embryos at a comparable stage.

The results for a series of single eight-cell embryos and single blastocysts are given in Figs 2 and 3. The variances of the distributions are relatively small, and are not consistent with a bimodal distribution, corresponding to two populations of equal size with modes differing by a factor of two. Even the distributions for HGPRT activities alone (Figs 2*b* and 3*b*) are not consistent with such a model. The mean levels of activity are in good agreement with those obtained by Epstein³ using another assay on groups of embryos. At the eight-cell stage, the earliest stage at which single-embryo assays are practicable, the activity levels are low, and may merely reflect the levels of maternal mRNA. By the blastocyst stage, however, activity levels are 30-fold higher than at fertilisation and embryonic gene action at the HGPRT locus is established by Epstein's work³. We therefore conclude that some form of dosage compensation operates in most, if not all, cells of the pre-implantation blastocyst. The trophectoderm constitutes most of the cells of the blastocyst but further analysis is required to establish whether dosage compensation operates within the cells of the inner cell mass as well as in those of the trophectoderm.

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MARILYN MONK
HASUMATI KATHURIA

MRC Mammalian Development Unit,
Wolfson House,
4 Stephenson Way,
London NW1, UK

Received 13 July; accepted 18 July 1977.

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Development to term of chimaeras between diploid parthenogenetic and fertilised embryos

PARTHENOGENETIC mouse embryos have the potential for extensive cellular proliferation as well as differentiation into various cell types^{1–3}. But this potential has been realised only when parthenogenetic embryos have been transferred to extrauterine sites¹, and in spontaneously occurring ovarian teratomas and teratocarcinomas of parthenogenetic origin^{2,3}. The development of mammalian parthenogenetic embryos *in utero* is restricted, with no conclusive evidence that they can develop to term^{4,5}. Several hypotheses have been proposed to account for their poor development. For example, deleterious recessive genes may affect the viability of their cells, possibly because of their extensive homozygosity^{2,5}, or disorganised growth and limited life span may result from anomalies of cellular interactions⁵. But the extensive cellular proliferation and differentiation of parthenogenetically derived cells which occurs in extrauterine sites is not entirely consistent with

these explanations, and indicates that parthenogenones probably have a relatively stable genetic constitution. Indeed, these studies stress the likely importance of cellular environment for cytodifferentiation, provided in this instance by the extrauterine host tissue. There is a precedent for supposing that if the environment is critical for cytodifferentiation, parthenogenetic cells should be able to form chimaeras with cells derived from fertilised embryos⁶. Teratocarcinoma cells^{7,8} and cells carrying known lethal alleles^{9,10} can develop into viable chimaeras when aggregated with cells from normal embryos. Previous attempts to achieve development to term of aggregation chimaeras between parthenogenetic and fertilised embryos were apparently unsuccessful¹¹. We have introduced inner cell masses (ICMs) from diploid parthenogenetic embryos into intact fertilised mouse blastocysts, and we report here the development of a chimaera to term.

Mouse oocytes isolated 18 h after injection of human chorionic gonadotrophin for superovulation from (C57BL × CBA) F₁ females were activated *in vitro* in medium lacking Ca²⁺ and Mg²⁺ salts^{5,12}, and the diploid parthenogenetic embryos allowed to develop to the blastocyst stage in culture⁵. Of 397 activated eggs 37 (14%) had one pronucleus and the second polar body; 160 (60.6%) had two pronuclei without the second polar body; eight (3%) had one pronucleus without the second polar body, and 59 (22.3%) went into immediate cleavage. The overall activation frequency was 66.5%. The embryos were homozygous for the isozyme of glucose phosphate isomerase (GPI), *Gpi-1^b/Gpi-1^b* and carried coat colour and eye colour genes of the C57BL and CBA strains. Of the 160 activated eggs with two pronuclei without the second polar body, 81 (50.6%) reached the blastocyst stage. We obtained 43 ICMs from the blastocysts and were able to transfer 20 to recipient blastocysts. ICMs were isolated by a technique involving exposure of blastocysts to the calcium ionophore A23187. The parthenogenetic blastocysts were exposed to A23187 at the final concentration of 2 × 10⁻⁵ M for 20–30 min. This caused selective lysis of trophectoderm cells, leaving the ICM cells intact (M.A.H.S., D. Torchiana and S.C.B., in preparation). Individual ICMs were introduced microsurgically¹³ into blastocysts on the 4th day of pregnancy obtained by matings of *Gpi-1^a/Gpi-1^a* albino CFLP random-bred mice (Anglia Laboratory Animals Ltd). After 3–4 h culture at 37 °C, blastocysts were transferred to the uterus of d3 pseudopregnant CFLP mice previously mated to proven sterile vasectomised males (the day when vaginal plug was found was d1 of pseudopregnancy). Four blastocysts were transferred to each of the five recipients.

One female examined on d9 of pregnancy contained four implantation sites. Four apparently normal early somite embryos were recovered. The embryos and their respective ectoplacental cones were separately typed for GPI¹⁴. All four embryos were chimaeric, whereas the ectoplacental cones only contained the *Gpi-1^a* isozyme band. Two embryos probably contained 40–50% contribution from parthenogenetic cells, whereas the other two contained between 5 and 30% contribution from parthenogenetic cells. This assessment was made subjectively from the intensity of staining *Gpi-1^b* and *Gpi-1^a* isozyme bands (Table 2). The other four females were autopsied on d19 of pregnancy. Three of them were not pregnant, having no evidence of implantation sites. One female contained two embryos (Fig. 1*a*). The smaller embryo was dead, although morphologically an apparently normal male. This embryo showed pigmentation of the sclera and iris and had probably survived up to d15–17 of pregnancy. The larger embryo was alive at autopsy, and initiated spontaneous respiratory movements. Attempts were made to foster the foetus, but it was unfortunately not accepted by the foster mother. Anatomical examination of this embryo showed it to be an apparently

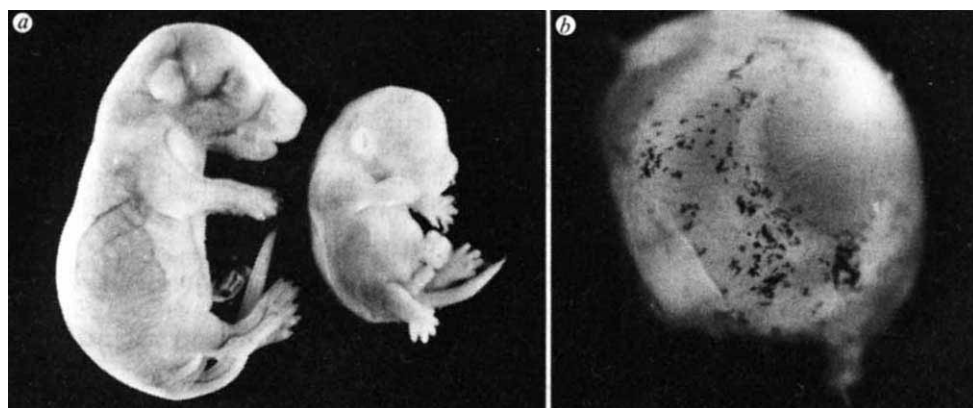


Fig. 1 *a*, Chimaeric embryos isolated from d19 pregnant female. The larger embryo was alive at autopsy and was an apparently normal female. The smaller embryo was dead and had probably developed until d15–17 of pregnancy and was an apparently normal male. *b*, An eye dissected from the larger embryo showing pigmentation of the choroid. The iris and retina were also pigmented.

normal female. The eyes were dissected out and showed pigmentation of the choroid (Fig. 1*b*) and iris, as well as the retina. All major organs from the two embryos were typed for GPI and found to be chimaeric. Between 40 and 50% of the cells in the smaller, dead embryo were of parthenogenetic origin, while in the live embryo the contribution was considerably smaller, being in the region of 10–25% (Table 1).

function normally^{15,16}. Similar cellular interactions between parthenogenetic and normal fertilised cells may also ensure cooperation of parthenogenetic cells in normal development.

These results suggest that it should be possible to obtain mature adult chimaeras. This would enable us to examine whether the parthenogenetically derived cells are fully differentiated in these chimaeras, and to examine whether they contribute to the normal functioning of all tissues and

Table 1 The contribution made by parthenogenetic cells in chimaeras assessed from GPI typing

Embryo no.	Day of pregnancy at autopsy	Description of embryos	Embryo (excluding membranes)	Ecto-placental cone	Tissues examined for parthenogenetic cells (%)*									
					Blood	Liver	Lung	Brain	Heart	Gonad	Kidney	Amnion	Eye	Carcass
1	d9	10–15 somite stage	40	ND	—	—	—	—	—	—	—	—	—	—
2	"	"	5	ND	—	—	—	—	—	—	—	—	—	—
3	"	"	50	ND	—	—	—	—	—	—	—	—	—	—
4	"	"	30	ND	—	—	—	—	—	—	—	—	—	—
5	d19	d15–17, male dead	—	—	NE	NE	NE	NE	NE	40	NE	NE	50	50
6	d19	d19, female live	—	—	25	10	15	10	10	15	10	15	15	15

ND, not detected; NE, not examined, tissue unsuitable.

*Values represent subjective assessment from GPI analysis based on the time of appearance of the *Gpi-I^a* and *Gpi-I^b* bands and the relative intensities of the two bands¹⁴.

These results demonstrate that cells from diploid parthenogenones, in addition to their potential for proliferating and differentiating in ectopic sites, can participate with cells from fertilised embryos in the development to term of an apparently normal chimaeric embryo. We tentatively suggest that a large number of such chimaeras may be able to develop to term when the parthenogenetic contribution is probably not more than 20% of the total cell population in the individual tissues and organs. The survival and integration of parthenogenetic cells in such chimaeras is probably largely influenced by the environmental conditions determined by the cells from the fertilised embryo, in contrast to the extensive but haphazard cell growth and differentiation which occurs when parthenogenetic and fertilised embryos are transferred to ectopic sites¹. The exact nature of this environmental influence remains unclear, but there are several probable ways in which such influences may be mediated. For example, exposed sites at the cell surface and/or secreted molecules may compensate for any deficiencies in parthenogenetic cells, permitting their normal development and differentiation. This type of influence may be involved in the 'rescue' of androgen-resistant germ cells⁹. There is evidence for metabolic cooperation between genetically diverse cell types through permeable junctions which enables metabolically deficient cells to

organs including the germ cells. This study also demonstrates that the presence of a genetic or extragenetic contribution from the male may not be a prerequisite for the normal development and differentiation of mammalian cells. Experiments are in progress to establish the differentiation potential of both haploid and diploid parthenogenetic cells.

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Note added in proof: One apparently normal chimaeric male which is now 4-weeks-old, has been obtained after further transfers of six operated blastocysts. Similar chimaeras have recently been reported by Stevens *et al.*¹⁷.

M. AZIM H. SURANI
SHEILA C. BARTON

*Physiological Laboratory,
University of Cambridge,
Cambridge, UK*

*Department of Anatomy,
University of Cambridge,
Cambridge, UK*

MATTHEW H. KAUFMAN

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Amiloride-sensitive rheogenic Na^+ transport in rabbit blastocyst

FLUID and solutes accumulate in the blastocyst just before implantation by transport mechanisms associated with the trophoblast^{1–3}. This structure is a simple squamous epithelium that surrounds the embryo, and it is the first tissue to develop in the mammalian embryo⁴. We describe here electrophysiological experiments which demonstrate changes in transport functions of this epithelium in the rabbit a few hours before the expected time of implantation. At this time fluid is accumulating rapidly in the blastocoele⁵. The following results show that between 6 and 7-d post-coitum (p.c.) a transtrophoblastic, rheogenic, amiloride-sensitive Na^+ transport system develops. The term 'rheogenic' characterises non-neutral or current-generating transport process, as opposed to the term 'electrogenic' which is more general and characterises any process (diffusion potentials, electrokinetic phenomena, and/or rheogenic transport) which results in a change in potential across an epithelium⁶.

The electrical potential difference between the blastocoele cavity and the external medium, the transtrophoblastic potential difference (ΔV_t) was measured in 4-, 5-, 6- and 7-d p.c. rabbit embryos. The electrophysiological techniques used to make the measurements were the same as those used in studies on the oocyte⁷, and the methods of obtaining and culturing the rabbit embryos were the same as used in former studies⁸. The measurements were made while the blastocysts were maintained at 37 °C on the heated stage of a microscope with continuous monitoring of the pH. The ΔV_t did not change between pH 7.4 and 7.6 and the media were maintained between these values. The embryos were incubated in a modified F10 medium^{9,10} that consisted of Ham's F10 medium (Microbiological Associates) buffered with 0.02 M HEPES (Calbiochem) containing 20% foetal calf serum and adjusted to pH 7.4 with 1 N NaOH. As shown in Fig. 1 there is a large change in the ΔV_t between 4 and 7 d p.c. with most of the change occurring between 6.0 and 6.5 d p.c.

The membrane potential of the trophoblast cells which surround the blastocoele was also measured. Because these cells are extremely thin it proved difficult to obtain long term penetrations (> 1 min). But the data from 30 trophoblast cells from eight 5-d blastocysts and 51 trophoblast cells from 15 6–7-d blastocysts demonstrate a significant change in electrical potential from -34.0 ± 4 to -46.7 ± 3.1 mV. The basis for this change has not been examined.

The effect of the two extracellular coats on ΔV_t was examined. The mucin coat, which is found on the 4-d p.c. blastocyst, caused no alteration of potential when the microelectrode was placed inside it, but outside the trophoblast layer. The zona pellucida was likewise found to have no effect. It was removed from the 5- and 6-d p.c. embryo by Pronase (0.25%) without causing change in the ΔV_t .

The results obtained on the ΔV_t are at variance with those reported by Cross and Brinster for the 5-, 6- and 7-d p.c. rabbit blastocyst measured *in vitro* in Krebs' Ringer bicarbonate with

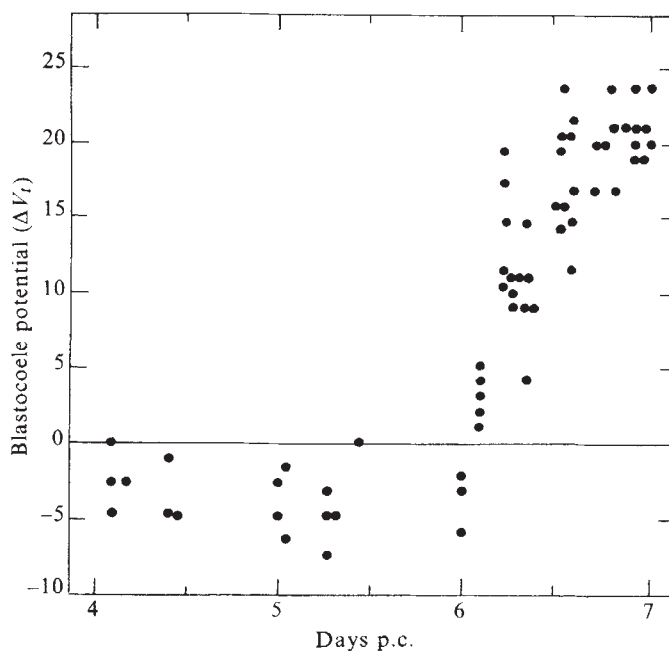


Fig. 1 Developmental changes in the transtrophoblastic potential difference (ΔV_t) measured in freshly collected rabbit blastocysts on days 4, 5, 6 and 7 p.c. The ΔV_t were measured at 37 °C in modified F10 medium supplemented with 20% foetal calf serum. The pH of the medium was 7.4.

glucose (KRBG) (-7.6 mV, -11.9 mV, -2.5 mV, respectively, blastocoele negative with respect to the medium) and for the 6-d p.c. blastocyst (-6.0 mV) in Eagle's medium with 20% foetal calf serum¹¹. Our measurements show that the ΔV_t in 4- and 5-d p.c. blastocysts is negative (-3.14 mV and -4.38 mV), but by 6.5-d p.c. the ΔV_t rises to $+14.5$ mV and at 7 d reaches $+21$ mV (Table 1). This change in polarity is found both in embryos cultured *in vitro* from day 5 and those allowed to remain in the uterus until day 6. Measurements were not attempted beyond day 7, the normal time for implantation, as the blastocysts become very fragile in culture beyond that stage.

In order to resolve the discrepancy with Cross and Brinster's results the effect of different media on the measured potential was examined. Medium F10 with 20% foetal calf serum for rabbit embryo culture was used routinely as it has been shown to be more effective than KRBG for long term culture^{9,10}. Cross and Brinster measured the ΔV_t throughout development in KRBG, but, as shown in Table 1, this does not account fully for the difference in measured values. We do find a significant reduction, however, in the ΔV_t of 6.5-d p.c. blastocysts when they are moved from F10 to KRBG. This reduction may relate to a reduction of energy available for ion transport, in Na^+ coupled amino acid or carbohydrate transport, or an alteration in membrane ion permeability. The data suggest that studies on blastocyst transport should be performed on identical age embryos to avoid developmental changes which will make results ambiguous.

The ΔV_t was also measured in KRBG after various ion substitutions. On day 5 p.c. the ΔV_t changes to a positive potential in Cl-depleted medium, whereas replacement of Na^+ with choline results in an increase in the ΔV_t . NaCl-free medium caused a reversal in polarity of the ΔV_t . The response of the ΔV_t is in the direction predicted by the electrochemical gradients for these ions⁸, and the results agree with those reported by Cross and Brinster¹¹. On day 6.5 p.c. Na⁺-free medium causes a larger decrease in the ΔV_t . Cl-depleted medium caused a slight increase in ΔV_t but this increase was not statistically significant.

Amiloride has been extensively used to analyse Na transport in epithelia^{12–14} and it is presumed to act by blocking passive Na entry into epithelial cells. No measurable effect of amiloride in the 5-d blastocyst in concentrations up to 1 mM in KRBG has been found. But, the ΔV_t of 6.5-d p.c. blastocysts is amiloride-sensitive

Table 1 Transtrophectodermal potential difference of the rabbit blastocyst

Medium	Age (d p.c.)			
	4	5	6.5	7
F10	-3.14 ± 0.77 <i>n</i> = 7	-4.38 ± 0.68 <i>n</i> = 8	$+14.5 \pm 1.0$ <i>n</i> = 26	$+21.0 \pm 0.8$ <i>n</i> = 15
KRBG		-3.8 ± 0.8 <i>n</i> = 10	$+6.0 \pm 2.1$ <i>n</i> = 8	
Na ⁺ -free KRBG		-9.1 ± 1.2 <i>n</i> = 8	-15.2 ± 3.2 <i>n</i> = 6	
Cl ⁻ -free KRBG		$+6.67 \pm 1.0$ <i>n</i> = 6	$+9.0 \pm 2.3$ <i>n</i> = 5	
NaCl-free KRBG		$+3.3 \pm 1.1$ <i>n</i> = 6		
Amiloride in KRBG (10 μ M)		-4.12 ± 0.37 <i>n</i> = 6	-3.25 ± 0.65 <i>n</i> = 8	-3.85 ± 0.9 <i>n</i> = 5

The electrical potential of the blastocoele is given in mV positive or negative ($\pm$ s.e.m.) with respect to the bathing medium. KRBG contains the following solutes (mM): 119 NaCl, 4.74 KCl, 1.71 CaCl₂, 1.19 KH₂PO₄, 1.19 MgSO₄, 7H₂O, 25 NaHCO₃ and 5.55 glucose. In Na-free medium, NaCl was replaced with equimolar choline chloride and NaHCO₃ was replaced with choline bicarbonate. Na₂SO₄ replaced NaCl, and K₂SO₄ replaced KCl in Cl-depleted medium. In NaCl-free medium, choline methyl sulphate replaced NaCl and choline bicarbonate replaced NaHCO₃. The osmolarities of the ionically substituted media were adjusted with sucrose, and the pHs were brought to 7.4.

and can be reduced in a dose dependent fashion to approximately -3 mV by raising the concentration of amiloride in the medium to 10 μ M. The response of the 6–7-d p.c. embryo to amiloride is maximum at 10 μ M, is directly proportional to the magnitude of the positive ΔV_i and is immediate. These findings provide strong evidence that the increase in ΔV_i seen between 5 and 7-d p.c. is the result of the onset of rheogenic Na⁺ transport. This mechanism is, in all likelihood, responsible for the increase in blastocoele Na and Cl which occurs during this period⁸, and Cross¹⁵ obtained evidence suggesting that Na and Cl influxes into the 6-d p.c. rabbit blastocoele may be coupled to each other. This hypothesis is consistent with our results on the 4 and 5-d embryo, but not with the positive ΔV_i that begins at 148 h p.c.

The current generating mechanism reflects the movement of a charged species across a barrier which has electrical resistance. The potential generated across the epithelium is directly proportional to the resistance of the epithelium and the rate at which ions are actively moved across the barrier. The change in potential which is observed between 6 and 7 d p.c. may, therefore, be caused by either an increase in the resistance of the trophoctoderm or an increase in Na⁺ influx into the blastocoele, Cross¹⁵ calculated a resistance of 2,742 Ω cm² for the 6-d blastocyst from short-circuit current measurements. Because of microelectrode tip resistance, the large surface area of the 6-d blastocyst and the greatly decreased total resistance of the trophoctoderm, we have not been able to measure the resistance of highly expanded blastocysts on day 6. Blastocysts on day 4 p.c. are small and have a large total resistance compared to the microelectrode's tip resistance. Measurements of the electrical resistance of eight blastocysts on day 4 p.c. indicate that the rabbit trophoctoderm has a resistance of $3,117 \pm 400 \Omega$ cm². These data, as well as 5–8 tight junctions on the apical surface of the trophoblast cells^{16,17}, and the impermeability of the trophoctoderm to lanthanum¹⁶, support the idea that the trophoctoderm is a tight epithelium. Because of the uncertainties in the measurements of resistance on the 6 and 7-d blastocyst a definitive statement about the mechanism(s) causing rheogenic Na⁺ transport cannot be made. The acquisition of an amiloride-sensitive ΔV_i that correlates in time with changes in the polarity and magnitude of the ΔV_i is suggestive of a developmental change in ionic transport mechanisms. Future studies will attempt to establish the relationship between the Na⁺ transport process described here and the ouabain sensitive Na transport system located on the juxtacoelomic surface of the rabbit trophoctoderm (J.D.B., R.M.B. and Lechene, C. P. unpublished).

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R. DOUGLAS POWERS*
R. MICHAEL BORLAND
JOHN D. BIGGERS

Department of Physiology and
Laboratory of Human Reproduction and
Reproductive Biology,
Harvard Medical School,
45 Shattuck Street,
Boston, Massachusetts 02115

Received 24 August; accepted 7 October 1977.

*Present address: Department of Biology, Boston College, Chestnut Hill, Massachusetts 02167.

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Divergence of metabolic activation systems for short-term mutagenesis assays

POLYCYCLIC hydrocarbons such as benzo(a)pyrene (BP) are prevalent environmental contaminants³ and are increasingly suspect as human carcinogens. They are routinely used as tumour agents in laboratory animals¹ and in tissue culture assays². These compounds are produced by the incomplete combustion of carbonaceous material such as fossil fuels used for transportation and industrial energy production. They are biologically inactive as parent compounds and require metabolic activation in tissue to produce a tumorigenically active species of the molecule. Activation is accomplished through the drug metabolising monooxygenase enzymes containing cytochrome P450 (refs 4–6), and activate the parent carcinogen to an intermediate epoxide^{7,8} which readily alkylates cellular macromolecules.

Figure 1 shows a compilation of known BP metabolites and those in brackets which have not yet been isolated or sufficiently characterised. Epoxide intermediates (4,5-; 7,8-; 9,10-) are further metabolised by microsomal epoxide hydrolases to produce diol derivatives or undergo non-enzymatic ring opening to produce phenols which are labile and form quinones as oxidation products. The metabolite profiles for benzo(a)pyrene in numerous species and tissues such as skin has not produced unique metabolites that are not present in resistant tissues. Quinones are formed both enzymatically and non-enzymatically, and their role in tumorigenesis remains uncertain. Therefore, susceptibility to tumorigenesis by these chemicals may be a function of metabolite kinetics since the rate of formation and removal of the activated carcinogenic species of benzo(a)pyrene may greatly influence the probability of the active molecules alkylating target site for carcinogenesis. There are at least three major enzymatic steps in this biochemical scheme—epoxidase, hydrolase and transferase to water soluble conjugates. Also there is considerable species variation within at least the first enzymatic reaction step⁹. Sims *et al.*¹⁰ showed that the 7,8-diol metabolite was remetabolised by the microsomal oxygenases to a 7,8-diol-9,10-epoxide, and this

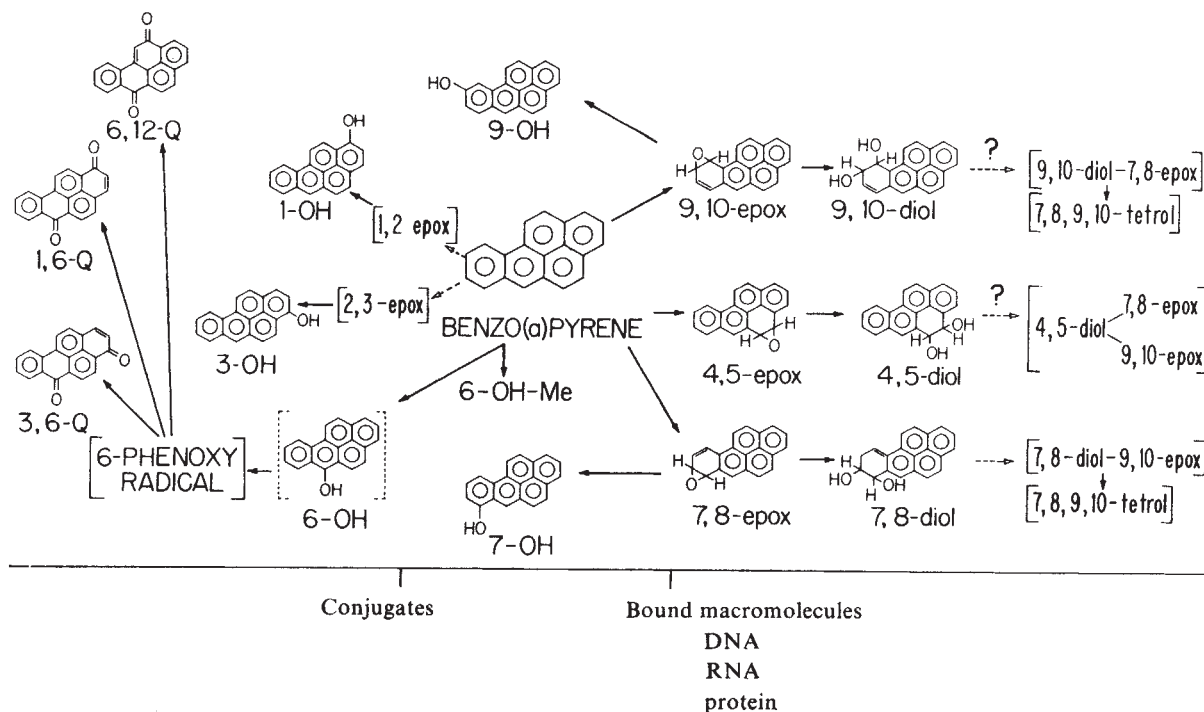


Fig. 1 Benzo(a)pyrene metabolites. Drawn structures represent isolated and characterised derivatives. 7,8-epox. and 9,10-epox. have been confirmed through isolation of their respective diols. Bracketed structures are possible metabolites. The 6-phenoxy radical is not stable, but readily seen by its electron-spin resonance signal²³.

6,12-Q, benzo(a)pyrene-6,12-dione
1,6-Q, benzo(a)pyrene-1,6-dione
3,6-Q, benzo(a)pyrene-3,6-dione
1-OH, 1-hydroxy-benzo(a)pyrene

3-OH, 3-hydroxy-benzo(a)pyrene
6-OH, 6-hydroxy-benzo(a)pyrene
7-OH, 7-hydroxy-benzo(a)pyrene
9-OH, 9-hydroxy-benzo(a)pyrene
6-OH-Me, 6-hydroxymethyl-benzo(a)pyrene
9,10-epox., benzo(a)pyrene-9,10-oxide
4,5-epox., benzo(a)pyrene-4,5-oxide
7,8-epox., benzo(a)pyrene-7,8-oxide
9,10-diol, 9,10-dihydrodihydroxy-benzo(a)pyrene
4,5-diol, 4,5-dihydrodihydroxy-benzo(a)pyrene
7,8-diol, 7,8-dihydrodihydroxy-benzo(a)pyrene

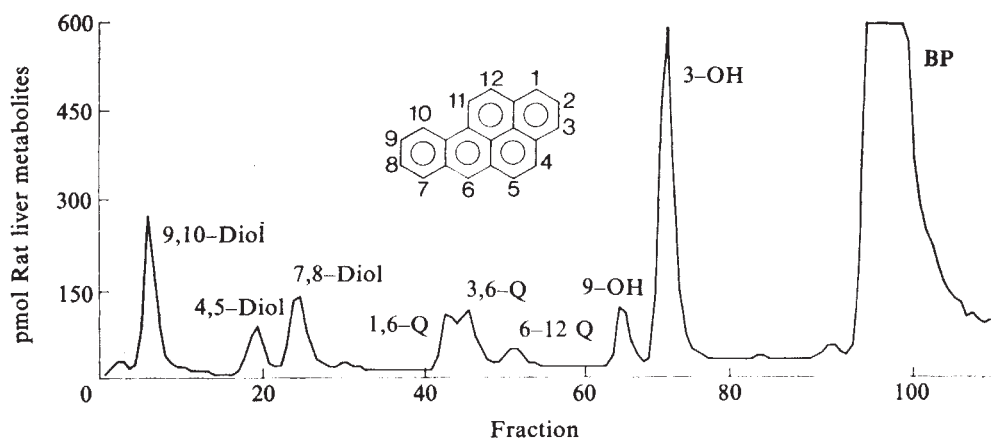
intermediate seems to be the major DNA binding metabolite along the pathway of benzo(a)pyrene metabolism¹¹⁻¹³.

If the diol-epoxide intermediate is one of the necessary requisites for development of the malignantly transformed state, then it will be critically important to compare metabolite patterns in susceptible and resistant tissues, species and cells. This may help to explain the variance in biologic susceptibility to polycyclic hydrocarbons. As there is a marked quantitative variation between liver microsomal metabolism of rat, mouse and hamster¹⁴ there is the strong suggestion that short-term *in vitro* mutagenesis assays either bacterial¹⁵ or mammalian cell¹⁶ which routinely add rat liver microsomal preparation for carcinogen activation may be producing mutagenesis data not

extrapolatable to other species, including man. To test this hypothesis, we have compared benzo(a)pyrene metabolism by liver microsomes, both rat and hamster, against metabolism by hamster embryo cells and hamster embryo cell microsomes with the use of high-pressure liquid chromatography.

To determine the uniformity of the metabolic pathway liver microsomes were prepared from male Sprague-Dawley rats weighing 125-150 g and Golden Syrian hamsters (Charles River, Boston, MA) as previously described¹⁷. Hepatic monooxygenases were induced by i.p. injection of 5 mg of methylcholanthrene in 0.5 ml of corn oil 40 h before killing. Primary cell cultures were prepared from hamster embryos¹⁴ and secondary cultures ($8-10 \times 10^6$ cells

Fig. 2 Rat liver microsome metabolism of benzo(a)pyrene. High-pressure liquid chromatography. Metabolite separation was performed with a Chromatronix 3500 high-pressure liquid chromatograph (HPLC) fitted with a DuPont 1-m ODS Permaphase column. Elution was by reverse phase, using a methanol-water gradient and ¹⁴C-derivatives as internal standards as previously described¹². Column pressure was 500 pounds per square inch and oven temperature was 50 °C. Effluent was monitored through an 8 µl flow cell on a DuPont 835 multiwavelength UV-fluorescence spectrophotometer with a 250-390 nm transmission filter. The gradient sweep time was 30 min. Fractions (0.2 ml) were collected and measured for radioactivity in a Searle Mark III scintillation counter using Aquasol (New England Nuclear, Boston, MA) as the counting solution. Abbreviations as in Fig. 1 legend.



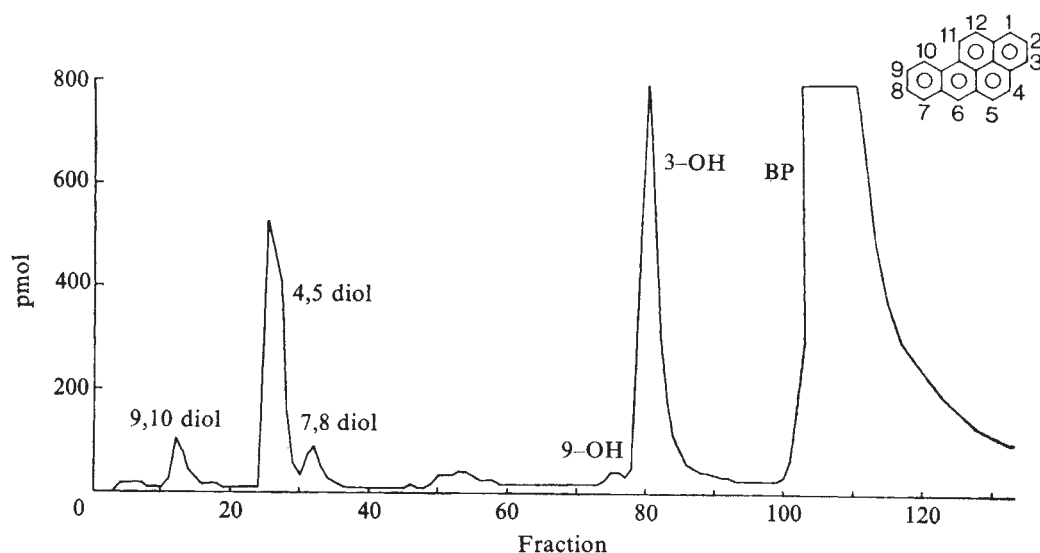
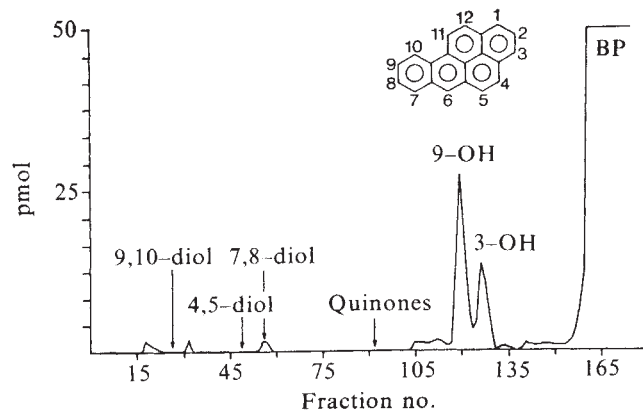


Fig. 3 Hamster liver microsomal metabolism of ^3H -benzo(a)pyrene. HPLC analysis of ethyl acetate extractable metabolites. Abbreviations as in Fig. 1 legend.

per 100 mm dish $\times$ 10 dishes) were incubated with ^3H -benzo(a)pyrene for 24 h and the cells and medium extracted into ethyl acetate for metabolite analysis. The microsomal cell pellet (100,000g) was prepared by scraping the cells from the dishes and gently lysing in a Potter-Elvehjem homogeniser in Tris-Cl buffer pH 7.5. Microsomes were suspended in Tris-sucrose buffer pH 7.5 and incubated for 30 min at 37 °C. The incubation contained in 1 ml: 15 μmol Tris-chloride buffer pH 7.5; 100 nmol ^3H -benzo(a)pyrene (specific activity 200 mCi mmol $^{-1}$) 0.35 μmol NADPH; 3 μmol MgCl $_2$, and 100 μg protein. Metabolism was halted by the addition of 1.0 ml acetone and the organic soluble metabolites from 10 incubations extracted into ethyl acetate for analysis by HPLC as previously described¹⁸.

Figures 2-5 clearly show marked differences in the hydroxylated products formed between rodent liver microsomes and intact or lysed cells. This profile variance suggests the monooxygenase system shows perturbation in the metabolic attack on different regions of the molecule. Rat (Fig. 2) and hamster liver (Fig. 3) microsomes show metabolite patterns containing three diols (9,10-; 4,5-; 7,8-dihydro-dihydroxy-BP) three quinones (1,6'- 3,6' 6,12-BP quinone) and two phenols (9-OH; 3-OH-BP) using this chromatography system. Liver contains the highest level of drug metabolising enzymes but is not a major target-site for polycyclic carcinogens. Hamster liver produces almost exclusively 4,5-diol while it forms a lesser component of rat liver metabolism.

Fig. 4 Metabolism of ^3H -benzo(a)pyrene by hamster embryo cell microsomes. HPLC analysis of ethyl acetate extractable metabolites. Abbreviations as in Fig. 1 legend.



In contrast, microsomes from hamster cells (Fig. 4) either produce insignificant amounts or further metabolise diols and quinones while forming 9-hydroxy as the major metabolites with less amounts of 3-hydroxy. Conversely, BP metabolism in intact hamster cells (Fig. 5) reverses the metabolite pattern. The 9,10- and 7,8-diols are the major components with only trace amounts of phenols. It would seem intact cells produce predominantly precursor diols for formation of the highly reactive diol-epoxides. Disruption of the cells clearly alter metabolite ratios indicating a requirement for spatial orientation of the enzyme complex within the microsomal membrane. Also, intact cells contain the cytoplasmic conjugates that are important for removal of reactive tumorigenic and toxic intermediates to water

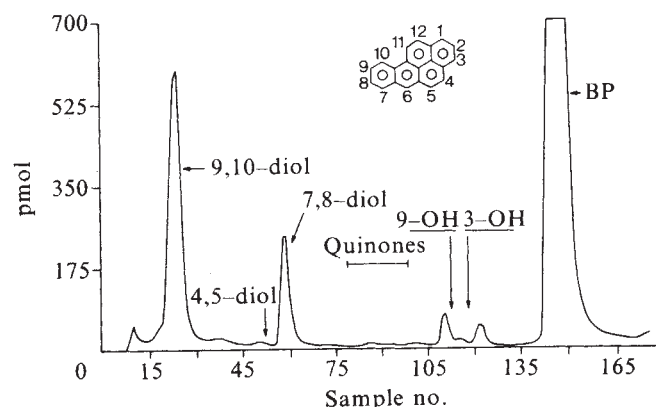


Fig. 5 Intact hamster embryo cell metabolism of ^3H -benzo(a)pyrene. HPLC analysis of ethyl acetate extractable metabolites. Abbreviations as in Fig. 1 legend.

soluble excretion products^{19,20}. The conjugating enzymes become diluted or lost during cell disruption and microsome preparation.

If activation of specific regions of the carcinogen molecule is more important for tumorigenesis than other regions of the molecule, then it is important that monooxygenase enzymes added to short-term *in vitro* mutagenesis assays reflect as closely as possible the enzyme activities in an *in vivo* situation. False positives or negatives which occur in bacterial assays may be simply a function of using a mammalian activation system to produce changes in bacterial biochemistry. *In vitro* assays utilising intact cell-feeder layers as activation systems for producing reactive

carcinogens for the test cell cultures seem to be the closest for *in vivo* comparisons^{17,18}.

Species resistance and susceptibility may be determined by which activation pathway is followed. The route, once selected, may in turn decide the kinetics of formation and disappearance of the activated carcinogenic species of the molecule and dictate whether that given species is a significant risk to polycyclic hydrocarbon induced tumorigenesis. Research supported by the National Cancer Institute (Yol-CP-50200) and the Department of Energy under contract with Union Carbide Corporation.

JAMES K. SELKIRK

Biology Division, Oak Ridge National Laboratory,
Oak Ridge, Tennessee 37830

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Identification of non-proliferating cells in melanoma B16 tumour

THE detection of growth fraction and the characterisation of non-proliferating cells are extremely important in human tumour therapy^{1,2}. Cytological criteria for individual objective discrimination *in vivo* between non-proliferating and proliferating cells with the same DNA content have not been previously available³. It has been shown recently, *in vitro*, that the transition from the non-proliferating to proliferating state (G0-G1) of WI-38 cells—human diploid fibroblasts—can be objectively characterised in the intact cell by flow microfluorimetry and geometric-densitometric texture analysis⁴. Similar differences in nuclear morphology and chromatin conformation between proliferating G1 and non-proliferating 'G0+Q' cells are reported here for the first time for tumour cells *in vivo*, by parallel utilisation of objective image analysis and multiparameter laser flow microfluorimetry, both correlated with autoradiography.

The experimental details have been published elsewhere⁴⁻⁶. Basically, the B16 melanoma tumour was grown in the foot pad of C57 mice: a suspension of 3×10^5 tumour cells was grafted s.c. into the foot pad of a 3-month-old mouse and 10 d later the tumour was excised. Triplicate smear preparations of tumour tissue were Feulgen-stained, using 1 M HCl at 60 °C for 10 min⁵, and image analysis of the microscopic slides was performed using a Quantimet 720-D automated image analyser (Cambridge Corp, New York).

The following parameters were measured, as previously described^{5,6}, for each nuclear image: integrated optical

density (IOD, proportional to DNA content), area, perimeter, projection, form factor (area divided by the square of the perimeter), average optical density (IOD divided by area), and mean bound path (area divided by projection).

Figure 1 shows a two-dimensional plot of about 100 tumour nuclei. The ordinate corresponds to the integrated optical density while the abscissa gives the average optical density (A_{av}). The IOD histogram reveals a distribution typical of a logarithmically growing cell population with a large distinct peak, resembling cells with the same DNA content (G0+G1). On the abscissa the A_{av} histogram of

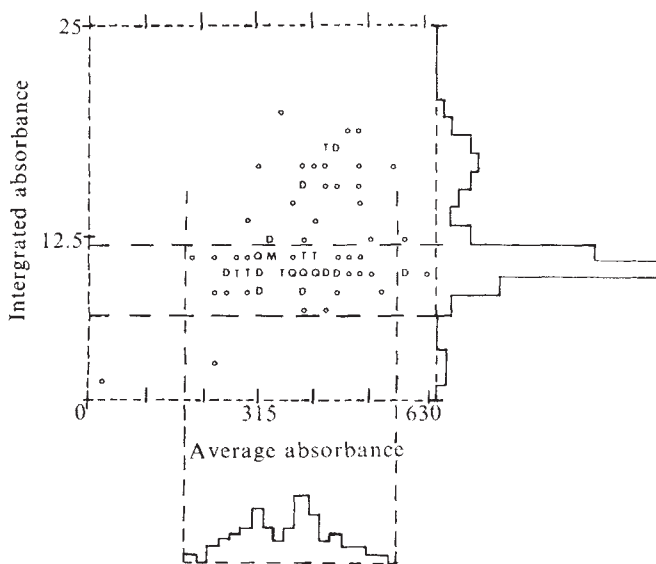


Fig. 1 Scattergram of Feulgen-stained melanoma B16 nuclei in a smear preparation. The integrated optical density of each nucleus is presented over its average optical density (both in arb. units). + = 1 cell, D = 2 cells, T = 3 cells, Q = 4, M = 5 or larger. The rectangle bound by the dashed lines containing the G0+Q and G1 cells. Fibroblasts contamination in our tumour cell suspension is less than 3%. The raw data were processed by various programs, loaded on a PDP11/40 computer⁶, for screening, purification, necessary statistics and computer display, as shown. Absorbance = optical density.

the same cells, present in the IOD unimodal peak, shows instead a bimodal distribution with two clearly separated peaks. By a striking analogy with our previous findings *in vitro* using WI-38 cells^{4,5} (the chromatin in the G1 cells is more dispersed than in G0 confluent cells) we may suppose that the two peaks correspond respectively to the proliferating G1 (dispersed chromatin) and non-proliferating G0 (condensed chromatin) tumour cells. This would result in a growth fraction (number of all proliferating cells/total number of cells) of about 0.65. Without any further direct proof, however, our identification of 'G0+Q' and G1 cells, as inferred from the clustering of the data in two sub-populations and the analogy with WI-388, could remain open to criticism. Therefore we conducted a continuous labelling experiment (Fig. 2) in order to achieve an independent estimate of the growth fraction using autoradiographic techniques.

One must be careful, however, in analysing progressive labelling data. Because the tumour is in balanced exponential growth, the proliferating and non-proliferating compartments are in a dynamic equilibrium. Therefore some proliferating P cells must subsequently become non-proliferating (G0+Q) and some G0 cells may resume proliferation. Also, some Q cells will become necrotic and disappear from the system. Thus, in order to determine the growth fraction from progressive labelling data, we must make a dynamic model⁷ (see Fig. 2), which best fits the

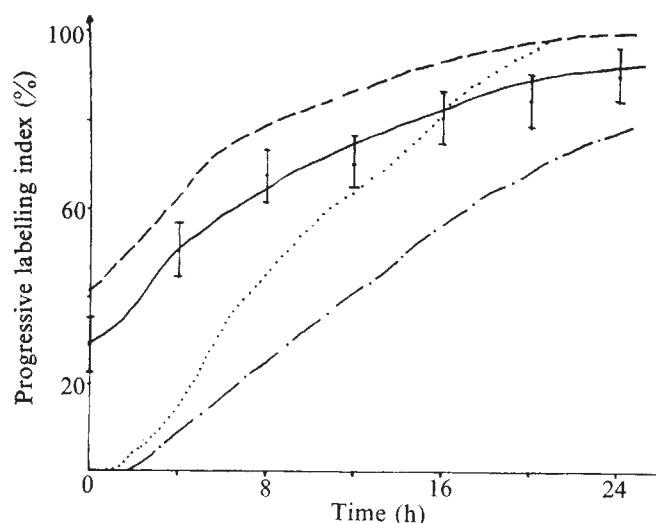


Fig. 2 Progressive labelling index of B16 melanoma cells. The curves show the predicted progressive labelling index of the B16 melanoma by a dynamic model⁷, which includes a proliferating P compartment, a reversible resting G0 compartment and an irreversible non-proliferating Q compartment. We have found that the model is fairly insensitive to the specific fraction of cells in G0 and Q and the continuous labelling curve depends only on the total (G0+Q) non-proliferating compartment. The solid line shows the predicted labelling index of the entire population. Superimposed are the experimental data points (●), with error bars. The dashed line shows the predicted labelling of the proliferating P compartment. The dotted-dashed line shows the labelling of the (G0+Q) non-proliferating compartment. The dotted line shows the fraction of the G1 compartment labelled. Note that there is not a great difference between the labelling of the (G0+Q) and G1 cells. This is a consequence of the balanced growth assumption. The experimental labelling indices (means and standard deviation), were determined by autoradiography of melanoma B16 tumours growing in C-57 black mice, at various hourly intervals after a single injection of ³H-thymidine (0.25 μ Ci g⁻¹ body weight), followed, at 4-h intervals, by six injections of ³H-thymidine (0.25 μ Ci g⁻¹ each). To determine the growth fraction, we fit a mathematical model⁷ to the data, by using as input the cell cycle phases residence times, doubling time (45.9 ± 1.5 h) and flash labelling index (0.27); by iteration, the predicted labelling index which best fitted the continuous labelling data are consistent with Q cells lost of 0.07 per h and a growth fraction of 0.68 ± 0.03 .

data with a (G0+Q = NP) non-proliferating compartment of about 32%. Since only cells in the proliferating P compartment may divide, at every division a constant fraction of P cells become NP cells. Because all P cells will eventually be labelled, this implies that cells in the non-proliferating compartment must eventually be labelled (Fig. 2), making it impossible to have direct evidence that all cells out of cycle by image analysis (Fig. 1) are also cells out of cycle by autoradiography, that is, unlabelled. Therefore, to make our discrimination of 'G0+Q' from 'G1' cells more than circumstantial, we analyse a cell suspension from the melanoma tumour by an independent method—multiparameter laser flow microfluorimetry^{4,9}. Unfixed cells were stained with acridine orange (AO), either directly as previously described^{4,10}, or after treatment with Triton and chelating agents⁹. Both procedures yield similar results (Fig. 3), showing either in the green⁴ or red emission⁹ a peak with lower fluorescence clearly separated from fluorescence distribution typical of log-phase population (that is, proliferating cells in G1, S, G2 and M phases).

Analysis of the two larger peaks, with different fluorescence, but with similar low angle scatter (size), sorted by a two-parameter fluorescence activated cell sorter, shows that, in our experimental conditions, both peaks contain only single tumour cells, with the same amount of DNA (diploid), undistinguishable by visual observation. Furthermore, 8 h after progressive labelling with ³H-thymidine, the

tumour cells with lower fluorescence show less incorporation of radioactive thymidine (non-proliferating compartment) than tumour cells with larger fluorescence ('G1'), compatible with the prediction of the model outlined in Fig. 2. For 12 different experiments, the fractions of non-proliferating tumour cells, as determined by a computer fitting to the experimental data¹⁰, range between 29% and 35% (Fig. 3 and Table 1). The agreement among autoradiography, laser flow microfluorimetry and image analysis in determining the fraction of non-proliferating cells in the melanoma tumour *in vivo*, is striking (Table 1).

Table 1 Percentage of cells in each cycle phase for the same melanoma B16 tumour cell population growing in C-57 black mice

	G0+Q	G1	S	G2+M
Autoradiography	32 $\pm$ 3	28 $\pm$ 10	27 $\pm$ 7	13 $\pm$ 4
Automated image analysis	34 $\pm$ 3	34 $\pm$ 2	24 $\pm$ 4	9 $\pm$ 1
Laser flow microfluorimetry	32 $\pm$ 3	35 $\pm$ 4	23 $\pm$ 4	10 $\pm$ 2

Determined by autoradiography, laser flow microfluorimetry and image analysis. The means and standard deviations have been obtained from up to 12 different experiments. Experimental details are given in the text.

These independent autoradiographic and FMF studies permit the identification, as 'G0+Q' and 'G1' compartments, of the two peaks in A_{av} distribution of melanoma cells with the same IOD (DNA) (Fig. 1), which corresponds to the two peaks in green fluorescence distribution of the same melanoma cell with similar size and diploid levels (Fig. 3). Specifically, lower A_{av} of the nuclear chromatin corresponds to higher binding sites for acridine orange in intact cells ('G1' cell), while higher A_{av} corresponds to lower binding sites ('G0+Q' cell). Calculating mean values of the optical parameters for each of the two cell subpopulations respectively in the 'G0+Q' and in the 'G1' peak, we get the results presented in Table 2. While the two tumour cell populations have the same DNA content, the average optical density and the form factor of the G1 cells are significantly ($P < 0.01$) lower than the respective values of the (G0+Q) cells. The increase in average horizontal projection and the resulting decrease in mean bound path (Table 2) in the face of both increasing area and perimeter, strongly suggest that the decrease in two-dimensional circular geometry (smaller form factor) is partially due to a significant increase in surface convolution—merely a change in shape to a regular but less circular geometry^{5,6}. Thus, 'G1' nuclei expose a bigger (with respect to 'G0+Q' cells) surface to intercalating dyes than to active enzymes, such as RNA polymerase, compatible with the frequently reported increase in template activity during G0-G1 transition of

Table 2 Comparison of integrated optical density (DNA content), mean bound path, average optical density and form factor of 'G0+Q' and G1 melanoma B16 tumour cells

	(G0+Q) cells	G1 cells
Integrated optical density (arb. units)	10,720 $\pm$ 1,270	10,800 $\pm$ 1,300
Average optical density (arb. units)	438 $\pm$ 67	287 $\pm$ 40
Form Factor (Undimensional)	(5.6 $\pm$ 1.2)10 ⁻²	(3.2 $\pm$ 1.4)10 ⁻²
Mean bound path (μ m)	4.06 $\pm$ 0.4	3.16 $\pm$ 0.4
Horizontal projection (μ m)	6.3 $\pm$ 1.2	11.6 $\pm$ 3.1

Cell assignment is obtained in the bimodal distribution of average optical density (A_{av}) of tumour cells with the same DNA content (IOD), but with A_{av} larger (G0+Q) and smaller (G1) than 360 arbitrary units (values are means $\pm$ σ).

different cell lines⁸. Furthermore, the scatter plot of form factor against average optical density (not shown) presents no overlap between 'G0+Q' and G1 tumour cells, that is, the same cell which presents a lower degree of chromatin condensation (Fig. 1, Table 2) shows also an increase of chromatin convolution (Table 2). A convincing and striking biological implication is that these phenomena seem to occur whenever a cell increases the metabolic activity of its DNA, during early G1^{3,6}, G1-S^{3,6}, and G0-G1^{3,5,8}.

Our results prove, therefore, that geometric and densitometric image analysis, as supported by multiparameter laser flow microfluorimetry, provide a simple optical method for objective individual identification of proliferating G1 and non-proliferating 'G0+Q' cells, with the same DNA content, in an *in vivo* system, such as melanoma B16 tumour growing in mice (Figs 1-3, Tables 1-2). The differences in the nuclear morphology can be interpreted in terms of increased chromatin dispersion and convolution in proliferating G1 cells^{3,5}, compatible both with the increased dye uptake^{6,9} (caused also by a larger amount of RNA in G1 cells) and with the changes in template activity and tertiary-quaternary structure observed in *in vitro* systems^{8,11}.

Finally, the possibility of determining, by these direct and fast means, the growth fraction¹⁻³ in intact tumour cell suspensions, *in vivo*, could become extremely useful in

human cancer chemotherapy, besides providing objective evidence for the existence of a different degree of chromatin superpacking^{4,5,8,11} for cells in the non-proliferating compartment, even in animal tumour systems. The sorting of homogenous 'G0+Q' and 'G1' tumour cells, will allow also further characterisation of the molecular mechanism controlling mammalian cells proliferation *in vivo*.

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C. A. NICOLINI*
W. A. LINDEN
S. ZIETZ
C. T. WU

Division of Biophysics,
Temple University Health Sciences Center,
Philadelphia 19140

Received 7 June; accepted 17 October 1977.

*To whom all correspondence should be addressed.

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Role of non-conventional natural killer cells in resistance against syngeneic tumour cells *in vivo*

IMMUNE reactions of 'conventional' T and B lymphocytes are generally thought to constitute major parts of any measurable, 'specific' tumour resistance against autologous tumours. There is no doubt that protective immunity can be induced against subsequently transplanted syngeneic tumours in experimental systems¹. But, there is only scanty evidence to suggest that conventional immune reactions can provide resistance against such tumour cells when transplantation is made into normal individuals. Yet, it is frequently found that even long-transplanted syngeneic tumour cells may require comparatively high numbers of cells to ensure tumour take in normal recipients. Mice failing to succumb to small numbers of tumour cells can frequently be shown to reject a second graft of the same tumour with similar vigour². Thus, natural resistance against tumours may occur with no display of classical, immunological memory. That such natural protective forces do exist against tumours has been claimed for many years³, but its underlying basis is poorly understood. Here, we present data indicating that naturally occurring killer cells may play a decisive part in providing resistance against syngeneic tumour cells *in vivo*.

Natural killer (NK) cells with the ability to kill several kinds of tumour cells in short-term *in vitro* assays are found in mice and in other species (see refs 4 and 5 and refs therein). These NK cells are most numerous in spleen and blood and are under autonomous control by stem cells in the bone marrow⁶; they develop in the absence of the thymus^{7,8} and do not (by various criteria) belong to either conventional B or T lymphocytes or the macrophage-monocytic lineage. They appear and disappear in a highly characteristic manner according to the age of the mouse^{7,8}. The level of NK activity in individual animals is under comparatively strict genetic control⁹, allowing the classification of inbred mouse strains into 'low' or 'high' NK type. It is therefore possible,

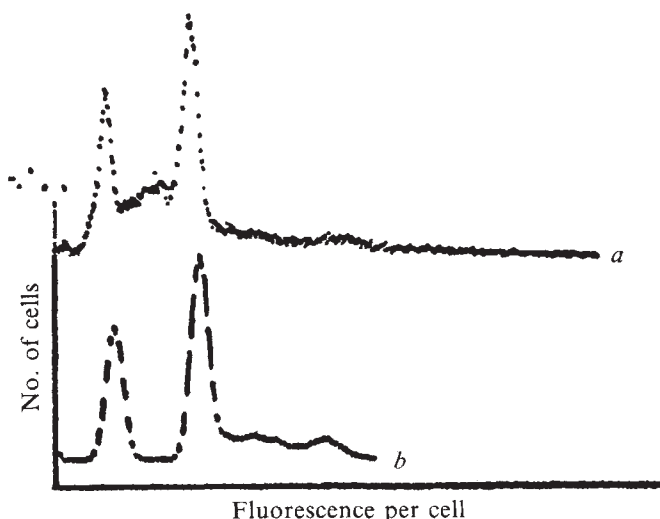


Fig. 3 Frequency distribution of green fluorescence per cell from a suspension of melanoma B16 tumour cells, obtained after 20 min of trypsinisation in 0.25% trypsin solution. Unfixed cells were stained directly⁴ with acridine orange at R (μM acridine orange per μM DNA) = 1.0; here the green emission was measured in order to monitor differences in chromatin DNA binding sites (which are known to be somewhat smaller in non-proliferating cells^{3,4,8}). Similar distributions were obtained after treatment with non-ionic detergent at pH 3.0 and exposure to chelating agents⁹: unfixed cells were stained with acridine orange⁹ and their total (or red) emission measured in order to take advantage also of differences in RNA⁸ (at substantially smaller levels in non-proliferating cells^{8,9}). In both instances, a simultaneous on line acquisition and reduction of low angle scatter and fluorescence was necessary in order to achieve discrimination (by selecting cells with larger scatter) of viable melanoma cells from necrotic cells and lymphocytes, which infiltrate the tumour. The experimental distribution (a) was obtained on a B-D fluorescence activated cell sorter on line with a PDP11/40, triggering on homogeneous cell populations of larger size (forward angle scatter). The best theoretical¹⁰ fit (b) to the data shown above gave a non-proliferating compartment G0+Q of 31%, with the proliferating compartment divided among G1 (36%), S (23%), and G2+M (10%) phases. The increased dye uptake for G1 tumour cells in respect to G0+Q is identical (about 2.45 times larger) to that previously reported for G0-G1 transition, *in vitro*^{3,4}. Fluorescence and low angle forward light scattering were measured either on a Cytofluorograf 4800A (Ortho Systems Co.) or a FACS-I Cell Sorter (Beckton-Dickenson), both on line with a PDP11/40 digital computer (up to one million cells were measured).

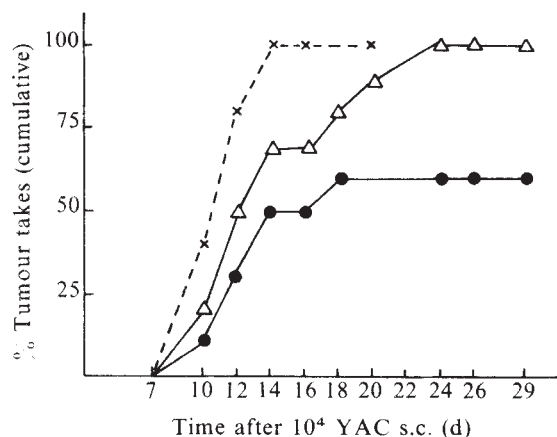


Fig. 1 Percentage tumour takes in animals inoculated with 10^4 YAC tumour cells subcutaneously. ●, (A/Sn $\times$ C57BL/6) F_1 mice irradiated with 750 R and reconstituted with (A/Sn $\times$ C57BL/6) F_1 anti-Thy serum + C treated bone marrow cells. △, (A/Sn $\times$ C57BL/6) F_1 irradiated with 750 R and reconstituted with (A/Sn $\times$ A.BY) F_1 anti-Thy serum + C treated bone marrow cells. ×, A/Sn control mice. 10 animals in first two groups, 6 in the last one. All animals with tumours died with tumours. For detailed conditions of transfer see ref. 10. A.BY and C57BL/6 mice have the same H-2 = H-2B. $0.02 < P < 0.05$ in χ^2 test comparing the two groups of marrow reconstituted F_1 mice. If data are pooled with data from Table 1 the differences in tumour takes observed between NK 'high' and NK 'low' F_1 hybrids are significant at $P < 0.001$.

using lethal irradiation followed by reconstitution with histocompatible bone marrow stem cells from low or high NK strains, to produce individual adult mice with distinct variation in NK activity but otherwise almost identical with regard to histocompatibility¹⁰.

Preliminary evidence suggests that there may be a positive correlation between the levels of NK activity (as measured *in vitro*) in a given individual and the degree of resistance of the same animal towards transplantation with a NK-sensitive tumour^{11,12}. Here, we show that tumour resistance *in vivo* is indeed positively correlated with the *in vitro* NK activity using two independent methods of changing NK activity *in vivo*. In the first experiments adult F_1 hybrid mice were irradiated and repopulated with bone marrow cells from high or low NK donors, both being histocompatible with the recipient as well as the tumour cells subsequently to be used. As mentioned above, it is already known that the eventual NK activity of the spleen cells of such repopulated animals is predetermined at the level of the bone marrow donor. To exclude *in vitro* artefacts being transferred into *in vivo* experiments, in rejection experiments we used the *in vivo* line of the YAC tumour line. YAC is a Moloney lymphoma of A/Sn genotype, known to be highly sensitive to NK cells when grown as an *in vitro* line¹³. The *in vivo* line which has never grown *in vitro* is also sensitive to NK activity although at a significantly lower

level. Figure 1 shows that A/Sn F_1 hybrids reconstituted so as to become either low or high with regard to NK activity express significant differences in resistance to YAC tumour cells. Thus, 'high' NK F_1 hybrids display significantly higher resistance than the 'low' hybrids when transplanted with 10^4 YAC cells. When the tumour dose was increased to 10^5 cells all mice did now succumb to tumour outgrowth with only minor differences in survival time (data not shown).

These results are consistent with the suggestion that NK cells play a decisive part in the rejection of histocompatible tumour cells. But, as these experiments were carried out in animals with irradiated but otherwise intact thymus it could be argued that the observed differences were determined by conventional T lymphocytes reacting against tumour-associated antigens in a 'normal', immune manner. Thus, a new series of experiments was set up but using animals thymectomised at an adult age before irradiation and marrow protection. The success of thymectomy was monitored by immunisation with polyvinylpyrrolidone (PVP), a T-independent antigen as well as with horse erythrocytes, a T-dependent antigen¹⁴ (data not shown). The animals were then transplanted with YAC cells and assessed for *in vivo* resistance to tumour outgrowth. Table 1 shows that thymectomy had no impact either on the level or on the pattern of tumour resistance: high NK marrow donors provided the host with eventual high resistance towards transplantation with YAC cells in the same manner as in the experiments depicted in Fig. 1. Thus, absence of immunocompetent T lymphocytes in the present system has no detectable role in providing *in vivo* resistance against syngeneic tumour cells.

NK activity changes more markedly with the age of the mouse than do B and T lymphocyte functions^{7,8}. Thus, NK activity appears abruptly around day 21 after birth and remains high until approximately 3 months of age followed by a rapid drop. Results from one system suggest a change in tumour resistance *in vivo* with age that would seem to parallel the NK activity¹². We have carried out experiments in our NK system to investigate this matter. Figure 2 shows the results of such an experiment carried out in a 'high' NK A/Sn F_1 -hybrid combination: there is a dramatic drop in resistance between 6 weeks and 6 months of age which corresponds with the reduction of *in vitro* NK activity^{7,8}. Experiments carried out in additional combinations using NK-sensitive tumour targets confirmed the observed age dependent pattern of *in vivo* resistance. Thus, these data also support the view that NK cells play a most decisive part in determining *in vivo* resistance against several kinds of syngeneic tumour cells.

As indicated above, results from other workers support our finding^{2,12}. Furthermore, NK cells are recruited when NK-sensitive tumour cells are being inoculated¹⁵ and can be isolated from tumour biopsies growing at locations where normally no NK cells would be detected¹⁶. Thus NK cells are apparently capable of migration to and destruction of sensitive targets in diverse regions of the body. Our findings may also shed some light on the confusing field of nonspecific immunotherapy of tumours.

Table 1 Intact *in vivo* resistance towards semi-syngeneic tumour cells in T-deficient animals

Expt	Recipient animals*	Bone marrow cells†	NK type‡	Tumour cells§	Frequency of tumour takes		
					Day 9	Day 20	Day 36
1	(A/Sn $\times$ 129/J) F_1	(A/Sn $\times$ 129/J) F_1	low	YAC, 10^3 , s.c.	0/8	6/8	7/8
		(A/Sn $\times$ C57BL/6) F_1	high	YAC, 10^3 , s.c.	0/8	0/8	0/8
2	(A/Sn $\times$ C57BL/6) F_1	(A/Sn $\times$ 129/J) F_1	low	YAC, 5×10^3 , s.c.	0/15	9/15	9/15
		(A/Sn $\times$ C57BL/6) F_1	high	YAC, 5×10^3 , s.c.	0/14	1/14	1/14

The experiments show a positive correlation between *in vivo* resistance and *in vitro* NK cell activity.

The differences in tumour takes observed comparing NK 'low' with NK 'high' F_1 mice are statistically significant for both experiments ($P < 0.001$ in χ^2 tests).

*Thymectomised at 1 month of age, irradiated with 750 R 24 or 26 d later. Reconstituted with anti-Thy + C treated bone marrow cells in Expt 1, with foetal liver cells in Expt 2 with genotype as indicated. Success of thymectomy was assessed by immunisation with PVP and horse erythrocytes respectively. Tumour cells grafted 3 weeks after reconstitution.

†Genotype of marrow or foetal liver cells. C57BL/6 and 129 have the same H-2 = H-2B.

‡NK activity of reconstituted mice as assessed *in vitro*¹³. (A/Sn $\times$ 129/J) F_1 spleen cells have normally at least 5 times lower NK cell activity compared with (A/Sn $\times$ C57BL/6) F_1 reconstituted mice.

§Transplantation subcutaneously of the H-2A tumour YAC at cell number indicated.

||Animals displaying tumour growth out of total number grafted. All tumour-bearing animals died with tumours.

Thus, two agents (BCG and *Corynebacterium parvum*) which are claimed to sometimes induce significant increases in tumour resistance *in vivo*^{17,18} have recently also been found to have grave impacts on NK cell activity^{15,19}. Interestingly, either enhancing or strongly suppressive effects on NK cells could be induced by the same agent depending on the conditions used for administration (Ojo, E., Haller, O. & Wigzell, H., unpublished work). It therefore seems possible that parallel measurements of NK activity *in vitro* may allow a controlled use of such immunotherapeutic substances in a manner previously not possible.

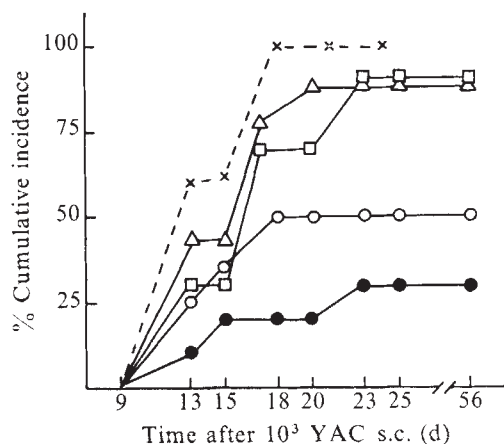


Fig. 2 Percentage tumour takes in (A/Sn x CBA/H)_{F1} mice of different ages inoculated with 10^3 YAC cells subcutaneously. (A/Sn x CBA)_{F1} is a 'high' NK combination¹¹. x, A/Sn control mice, 3 months of age. A/Sn mice are 'low' in NK activity. (A/Sn x CBA)_{F1} mice: ●, 31/2 weeks of age; ○, 6 weeks of age; □, 6 months of age; Δ, more than 9 months of age. Each group comprised of 8–10 mice. All animals carrying tumours died with tumours. $0.001 < P < 0.01$ in χ^2 test comparing the two groups of young mice (6 weeks or younger) with the two groups of old mice (6 months or older). If data from additional experiments (see text) are pooled, $P < 0.001$.

Our findings strongly support the suggestion that natural resistance against tumour outgrowth *in vivo* may be exerted largely via cells of a non-conventional type. Thus, a high positive correlation between NK cell activity *in vitro* and tumour resistance levels *in vivo* was found using two distinct methods of analysis. On the other hand, the cells normally considered to be the most important involved in cell-mediated immune reactions, that is, the thymus-dependent lymphocytes, failed to contribute to this *in vivo* resistance. If these results are found to be generally applicable, then this would suggest the existence, outside the conventional immune system, of a surveillance mechanism with a comparatively selective ability to recognise and destroy aberrant malignant cells *in vivo* through direct lytic contact mechanisms.

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Department of Immunology,
Uppsala University Medical School,
Box 582, S-751 23 Uppsala, Sweden

Department of Tumour Biology,
The Karolinska Institute,
S-104 01 Stockholm 60, Sweden

Department of Immunology,
Uppsala University Medical School,
Box 582, S-751 23 Uppsala, Sweden

O. HALLER

M. HANSSON
R. KIESSLING

H. WIGZELL

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Tumour cell lines induce interferon in human lymphocytes

VIRAL infection of cells induces synthesis and release of interferon which renders uninfected cells resistant to subsequent virus infection. Interferon production can also be stimulated by non-viral agents, such as microorganisms¹, substances of microbial origin and synthetic polymers². Moreover, interferon can be stimulated in lymphocytes by mitogenic lectins³, antilymphocyte sera⁴, viral antigens^{5–7}, PPD⁸ and exposure to allogeneic cells⁹. An antitumour effect of interferon and interferon inducers has been demonstrated *in vivo*^{10–12}. It has been postulated that interferon may act by directly inhibiting tumour cell growth¹³ and by stimulating host defence mechanisms^{14–16}. We report here that certain human cell lines are able to induce high levels of interferon on contact with human lymphocytes.

Purified human peripheral blood lymphocytes were cultured with confluent monolayers of various human cell lines. The antiviral activity of the supernatant was measured by inhibition of the cytopathic effect of vesicular stomatitis virus on monolayers of human foetal skin fibroblasts. Eight human tumour-derived cultures and two simian virus 40 (SV40)-transformed cultures induced high levels of antiviral activity when cultured with human lymphocytes (Table 1). The supernatant from cell lines cultured alone or from lymphocytes alone did not show antiviral activity (Fig. 1). All 12 human fibroblast lines derived from normal tissues, 13 out of 15 lines transformed by SV40, and five tumour-derived cell lines, were unable to induce interferon (Table 1). Most of the cell lines were checked for mycoplasma contamination at the time of the test: no correlation was found between the presence of mycoplasma and the induction of antiviral activity.

Mouse cell lines were also tested for their ability to induce interferon in human lymphocytes (data not shown). Several were positive, including some continuous cell lines and 5 out of 19 SV40-transformed cell lines. The interferon produced by cultivation of mouse cell lines and human lymphocytes was of human origin, as determined by its ability to inhibit viral replication in human cells and not in mouse cells: this result indicates that the lymphocytes in these mixed cultures and not tumour cells are responsible for interferon production.

The kinetics of interferon production in these experiments were similar to those observed after virus infection of lymphocytes (Fig. 1). Antiviral activity was detectable in the supernatant 4–5 h after the lymphocytes were added to the cell cultures and its titre increased up to 16–24 h. The final titres of antiviral activity varied according to the cell line and the lymphocyte preparation; however, the positive cell lines induced interferon production by each lymphocyte preparation and the negative cell lines never induced interferon.

The virus inhibitor in the supernatants of the mixed cultures meets all the criteria established by Lockart²⁴ to be considered as an interferon; it is species-specific, destroyed by trypsin, resistant to treatment at pH 2 and the active components can be eluted from a Sephadex G-100 column in two fractions corresponding to

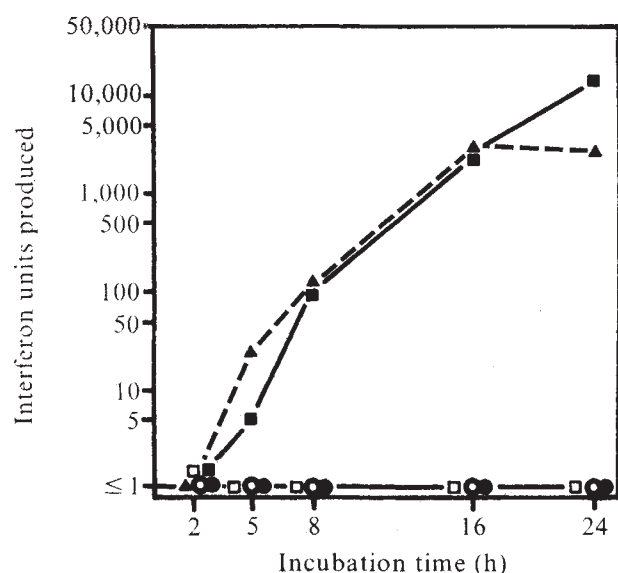


Fig. 1 Kinetics of interferon production from human lymphocytes infected with Newcastle disease virus or cultured with a human rhabdomyosarcoma-derived cell line. Human peripheral blood lymphocytes were isolated on a Ficoll-Hypaque gradient. Adherent cells were removed by two incubations (1 h each, 37 °C) on glass Petri dishes. The cells were resuspended at 10^7 ml $^{-1}$ in RPMI 1640 medium with 10% foetal bovine serum and 1 ml of medium containing cells was added to 16 mm wells (Linbro Disposo trays FB16-24TC) empty or containing a monolayer of human rhabdomyosarcoma-derived cells (cell line RDMC). The supernatants were taken at the indicated times, centrifuged at 1,000g for 30 min and tested for antiviral activity. Supernatants from Newcastle disease virus-infected lymphocytes were treated at pH 2 for 4 d and then neutralised before testing. The antiviral activity was expressed in terms of the cytopathic effect, 50% end point inhibition test. Serial dilutions (100 μ l) of the supernatant were added to the wells of microtitre plates (Microtitre II, Falcon) containing monolayers of human foetal skin fibroblasts. After overnight incubation the supernatants were removed and 50,000 plaque-forming units of vesicular stomatitis virus (Indiana strain) were added in a volume of 0.2 ml. The final evaluation of cytopathic effect was done after 48 h. Each assay included a standard interferon preparation (NIH human reference interferon G-023-901-527 with a titre of 20,000 units). Supernatants from: ●, lymphocytes alone; □, RDMC monolayer alone; ■, mixed culture lymphocytes and RDMC; ○, mixed culture lymphocytes and RDMC in presence of cycloheximide 100 μ g ml $^{-1}$; ▲, lymphocytes infected with Newcastle disease virus at multiplicity of infection of 10.

molecular weights of approximately 45,000 and 25,000. Inhibition of cellular protein synthesis, by the addition of cycloheximide to the lymphocyte-tumour cell culture, suppressed interferon production (Fig. 1). In addition to the antiviral activity, the supernatants from the mixed cultures inhibit cell growth and DNA synthesis in human cell lines and have an effect on the cytotoxic activity of human peripheral blood lymphocytes (manuscript in preparation). These activities, which have also been described with purified preparation of virus-induced interferons^{13-16,25}, in the supernatants from lymphocyte-cell line mixed cultures were positively correlated with the antiviral activity and were eluted in the same two peaks after gel filtration.

The stimulus for production of interferon by lymphocytes on incubation with cell lines is unknown. Contact between lymphocytes and cells seems to be required because supernatants from the cell lines are unable to induce interferon production by lymphocytes. When human cells were used as inducers, with the exceptions of two SV40-transformed cell lines, only tumour-derived lines were able to induce antiviral activity in human lymphocytes.

For two types of human tumours, melanoma and colorectal

adenocarcinoma, various cell lines from different patients were analysed. All four melanoma-derived cell lines tested induced interferon. Of the six colorectal carcinoma-derived cell lines, only two were positive. These two positive cell lines were derived from the same patient²⁰—line SW480 from the primary adenocarcinoma of the colon, and line SW620 from metastases in the lymph node of the same patient. The other four colorectal carcinoma cell lines tested did not induce interferon.

The allogeneic (or xenogeneic) antigens on the surface of the cells were probably not responsible for interferon stimulation. Preliminary experiments with autologous cells in humans and with syngeneic cells in a murine system have shown that interferon is produced even in the absence of allogeneic differences between the lymphocytes and the inducer cell line. Moreover, alloantigens are present on both inducing and non-inducing cell lines and in the presence of a strong allogeneic stimulus, as in mixed leukocyte culture, no antiviral activity was detected in the supernatant after 24 h of incubation (Table 1). The induction of interferon by an allogeneic stimulus in mouse mixed leukocyte culture has been reported to occur only after 4–5 d of stimulation and at a titre much lower than that observed in our experiments⁹. 'Tumour'

Table 1 Induction of interferon by various human cell lines co-cultured with human lymphocytes

Cell line	Ref.	Origin	Passages	Lymphocyte preparations tested	Units interferon induced*
6 preparations		Peripheral blood lymphocytes†		6	<1
2 strains		Foetal skin fibroblasts	6–40	6–12	<1
3 strains		Foetal lung fibroblasts	20–40	4–8	<1
2 strains		Brain	2–15	4	<1
7 strains		Skin fibroblasts	5–40	4	<1
4 lines	17,18	SV40-transformed fibroblasts	20–240	4–8	<1
9 lines	19	Brain, SV40-transformed	4–22	4	<1
SI054TR	19	Brain, SV40-transformed	32	4	193 $\pm$ 137
SI003 3WTR	19	Brain, SV40-transformed	8	4	125 $\pm$ 0
4 lines	20	Colorectal carcinoma	10–95	4	<1
SW620	20	Colorectal carcinoma	160–165	4	362 $\pm$ 137
SW480	20	Colorectal carcinoma	105–110	3	125 $\pm$ 0
SW690		Melanoma	80–90	5	850 $\pm$ 552
SW691		Melanoma	80–85	4	6,000 $\pm$ 3,000
SW843		Melanoma	30–40	4	244 $\pm$ 119
SW489		Melanoma	30–40	3	25 $\pm$ 0
RDMC	21	Rhabdomyosarcoma	150–200	32	3,494 $\pm$ 1,008
HT1080	22	Fibrosarcoma	110–115	4	<1
D98 (HeLa)	23	Cervical carcinoma	50–60	4	312 $\pm$ 165

Monolayers of the different cell lines have been co-cultured with various human lymphocyte preparations as indicated in Fig. 1.

*Antiviral activity was measured in the supernatants after 24 h of co-cultivation; values are means $\pm$ s.e.m.

†Equal volumes of allogeneic lymphocyte preparations from peripheral blood (10^7 cells ml $^{-1}$) were mixed and the supernatants collected after 24 h as in the lymphocyte-cell line mixed cultures.

antigens present on the surface of the inducer cells might be responsible for an immune stimulation of interferon. But, the ability of the lymphocyte preparations from all donors tested to produce interferon when cultured with inducer cell lines, the high titre of interferon produced and the early kinetics of production seem to differentiate the mechanism of interferon induction described in this study from other known systems of immune stimulation; for example, stimulation of primed human lymphocytes with various viral antigens induces only low levels of interferon (less than 100 units in most systems) after 5–7 d of stimulation^{6,7} and then only in lymphocytes from immune donors. Particular cell-surface characteristics in the positive cell lines might be responsible for the interaction with the lymphocytes and for their stimulation. The presence in the lymphocyte population of natural killer cells that are known to interact with and lyse some of the tumour cells during incubation²⁰ might be responsible for induction or release of the factor(s) responsible for interferon induction. The possibility that viruses present on the human cell lines are the inducers, although unlikely, cannot be excluded.

The ability of interferon to enhance specific and nonspecific cell-mediated cytotoxicity, as well as its inhibitory effect on tumour cell growth could be responsible for the antitumour effect *in vivo* observed with exogenous interferon and with interferon inducers. If, as observed with tumour-derived cell lines *in vitro*, tumour cells *in vivo* are able to interact with the host lymphocytes and induce interferon production, it is possible that this induction of endogenous interferon is one of the natural defense mechanisms of the host against tumour growth and invasion.

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GIORGIO TRINCHIERI*

DANIELA SANTOLI

BARBARA B. KNOWLES

Wistar Institute of Anatomy and Biology,
36th Street at Spruce,
Philadelphia, Pennsylvania 19104

Received 14 July; accepted 11 October 1977.

*Present address: Institut Suisse de Recherches Expérimentales sur le Cancer, CH-1066 Épalanges, S. Lausanne, Switzerland.

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Activation of human complement by glutaraldehyde-treated red cells

THERE is now much evidence that the classical complement pathway can be activated by substances other than immune complexes. Leonard and Thorne¹ found that precipitate formed between the polyanion, polyglutamic acid, and lysozyme was anticomplementary, although the site of action was not determined. Since then, many polyionic substances and complexes have been found to activate the classical complement pathway, namely polyinosinic acid², dextran sulphate^{3,4}, cellulose sulphate⁴, heparin–protamine complexes⁵, lipid A⁶, lysozyme–DNA complexes⁷, double-stranded DNA⁸ and C-reactive protein–polycation complexes⁹. Although activation by heparin–protamine complexes was found to take place in agammaglobulinaemic serum¹⁰, it is possible that the polyions do not react directly with C1, but that they bring about aggregation of IgG, which then combines with and activates C1. On the other hand, C-reactive protein attached to the surface of red cells can activate complement without involvement of antibody or IgG (refs 11, 12), as can certain oncoviruses¹³ and mitochondrial membranes¹⁴. It has also been shown that the complement component C1q will combine with glutaraldehyde-treated red cells and that the binding sites involved on the C1q molecule are probably those also involved in binding to immune complexes¹⁵. It has now been found that glutaraldehyde-treated red cells not only bind to C1q, but can also activate the complement system through the classical pathway, with no evidence of involvement of immunoglobulin.

Group O Rh-negative red cells were washed six times in saline and treated with glutaraldehyde followed by lysine, as previously described¹⁵. These cells were added to an equal volume of fresh serum and incubated at 37 °C for 2–24 h. Control samples contained 10 mM EDTA. Aliquots of the supernatant serum were then taken for assessment of complement activity. After incubation for 2 h, there was a reduction in complement haemolytic activity¹⁶ (CH₅₀) to 25–50% compared with that of the controls. Analysis of the complement components C2 (using a human homozygous C2-deficient serum), C4 (using ammonia-treated guinea pig serum¹⁶) and C3 and other late-acting components (using zymosan-treated guinea pig serum¹⁶) showed a progressive reduction in all components with almost complete loss of activity at 16–24 h. C3 breakdown products were also shown to be present in the serum after incubation for 6 h with glutaraldehyde-treated red cells, using the crossed immunoelectrophoretic method¹⁸, with an anti-serum containing anti-C3b, -C3c and -C3d. As Fig. 1 shows, there was almost complete conversion of C3 to C3c. Small

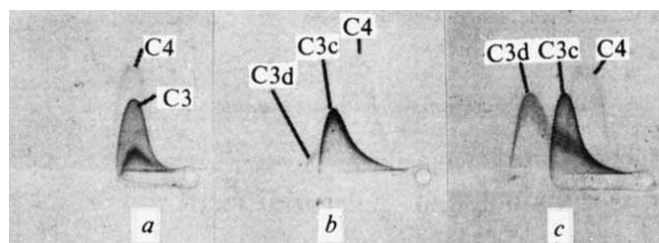


Fig. 1 Crossed immunoelectrophoresis of fresh serum after incubation for 6 h with glutaraldehyde-treated red cells, *a*, in the presence of 10 mM EDTA, and *b*, without EDTA. *c*, A serum aged for 4 d at 37 °C, used for identification of C3c and C3d precipitation lines. The antiserum contained antibodies specific for C3, C3c, C3d and C4.

amounts of C3d were present in the serum, but most was attached to the red cells, as shown by using an ^{125}I -labelled anti-C3d (ref. 17). The C1q content of the serum was reduced to barely detectable levels, as shown by single radial diffusion assay using anti-C1q.

These results are typical of those found with activation of complement through the classical pathway. It is improbable that the complement system was activated through IgG fixed to the red cells, as the cells were thoroughly washed before glutaraldehyde treatment, and only trace amounts of IgG could have been present on their surface. Activation of complement through IgG on the surface of membrane requires substantial amounts of antibody to be present; for instance, about 5,000 molecules of IgG anti-A are required on each red cell before complement components can be demonstrated to be present on the red cell surface¹⁹. Thus, it is unlikely that complement is being activated through IgG bound to the cells and it is more probable that the site for binding and activation of C1 is an integral part of the red cell membrane.

The ability of complement to be activated *in vitro* through the classical pathway by glutaraldehyde-treated red cells, as well as by oncovirus and mitochondria without the intervention of IgG, indicates that complement has the potential to act *in vivo* independently of other systems in the elimination of foreign membranous particles. Verhoef *et al.*²⁰ have provided evidence that the classical complement pathway can be activated by direct reaction with bacterial cell wall protein A, resulting in opsonisation and phagocytosis of the organisms.

N. C. HUGHES-JONES

BRIGITTE GARDNER

JANE ROWLANDS

MRC Experimental Haematology Unit,
St Mary's Hospital Medical School,
London W2, UK

Received 10 August; accepted 18 October 1977.

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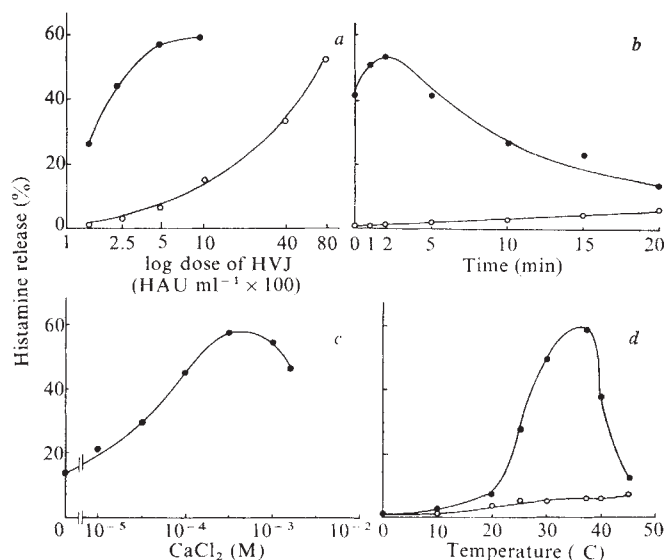


Fig. 1 *a*, Histamine release by HVJ and its potentiation by phosphatidylserine (PS). Isolated rat mast cells (1×10^5 – 2×10^6 cells) were incubated for 10 min at 37°C in varying concentrations of HVJ in PBS, with or without PS ($10 \mu\text{g}$). Total reaction volume was 1 ml. $\circ$, Histamine release by HVJ; $\bullet$, histamine release by HVJ and PS. Values for percentage histamine release are the means of experiments corrected for spontaneous release. *b*, Effect of PS on histamine release by HVJ in relation to the time of addition of PS. Cells were incubated for varying times at 37°C with HVJ (500 HAU ml^{-1}) in PBS, then PS ($10 \mu\text{g ml}^{-1}$) was added for a further incubation period of 10 min. $\bullet$, Histamine release by HVJ and PS; $\circ$, spontaneous release. *c*, Effect of Ca^{2+} on histamine release by HVJ and PS. Cells were incubated for 2 min at 37°C with HVJ (500 HAU ml^{-1}) in PBS containing CaCl_2 in varying concentrations, then PS ($10 \mu\text{g ml}^{-1}$) was added for a further incubation period of 10 min. $\bullet$, Histamine release by HVJ and PS; $\circ$, spontaneous release. *d*, Effect of temperature on histamine release by HVJ and PS. Cells were incubated for 2 min at varying temperatures with HVJ (500 HAU ml^{-1}) then PS ($10 \mu\text{g ml}^{-1}$) was added for a further 10 min incubation. $\bullet$, Histamine release by HVJ and PS; $\circ$, spontaneous release.

occurred frequently. This observation led to the theory that histamine release from mast cells was induced by virus, as well as by antigen from sensitised mast cells and by histamine releasers. The results reported here indicate that histamine release from mast cells was induced by direct exposure of cells to virus in the presence of Ca^{2+} at 37°C and that it was markedly enhanced by the addition of phosphatidylserine (PS), which is a selective enhancer³ of anaphylactic histamine release.

Mast cells were isolated from rat peritoneal fluid by the method as described before⁴ and suspended in a phosphate-buffered saline (PBS, 154 mM NaCl, 2.7 mM KCl, 0.9 mM CaCl_2 , 6.7 mM KH_2PO_4 – Na_2HPO_4 , pH 7.2 and 0.01% bovine serum albumin). HVJ-z strain was propagated in embryonated eggs and concentrated by a centrifugation⁵. The mast cell suspension was incubated with HVJ for 2 min at 37°C and then PS, sonicated in PBS, was added. After 10 min of incubation, the reaction was terminated by adding 2 ml of ice-cold PBS and the reaction mixture was centrifuged at 3,000g for 20 min. Histamine in the supernatants and precipitates was determined according to the fluorescence method of Shore *et al.*⁶. Lactate dehydrogenase (LDH) activity was determined by the method of Burch *et al.*⁷.

Incubation of HVJ suspension (final virus concentration, $8,000 \text{ HAU ml}^{-1}$) with mast cells for 10 min at 37°C induced histamine release accompanied by degranulation from the cells. With a concentration of 500 HAU ml^{-1} of HVJ, histamine release hardly occurred, but the addition of $10 \mu\text{g ml}^{-1}$ PS to such a cell suspension resulted in a marked histamine release (Fig. 1*a*), this release being almost com-

Histamine release from rat mast cells induced by Sendai virus

SENDAI virus (HVJ, haemagglutinating virus of Japan), a type 1 parainfluenza myxovirus, agglutinates certain types of cells and induces cell fusion¹. Previously² we reported on the degranulating activity in the hybrid cells derived from rat mast cells and Ehrlich ascites tumour cells using HVJ. During that work, it was found that histamine release accompanied by degranulation from unfused mast cells

plete within 5 min. The histamine release induced by HVJ and PS was dependent on the time at which PS was added. Figure 1b shows that when PS was added after a 2-min exposure of mast cells to virus, histamine release reached a maximum and then decreased on further exposure to virus. Phosphatidylcholine, phosphatidylinositol, phosphatidylethanolamine and phosphatidic acid were all ineffective in this respect.

Calcium ions were necessary for both the histamine release by HVJ and the potentiation by PS of HVJ-induced histamine release from mast cells. As shown in Fig. 1c, the effect of calcium increased with the increase of concentration from 10^{-5} M, reaching maximum at around 5×10^{-4} M. Histamine release induced by HVJ and PS in the presence of Ca^{2+} was dependent on temperature (Fig. 1d), the optimum being 37°C . At temperatures below 20°C and above 45°C , histamine release was inhibited.

The specificity of HVJ in histamine-releasing activity was examined. Adsorption of the virus to human erythrocytes resulted in complete inhibition of histamine-releasing activity of the virus. This activity was not, however, affected by repeated washing of the virus by centrifugation and resuspension. Histamine release from the mast cells induced by 500 HAU ml^{-1} HVJ was not accompanied by the release of LDH, but a prolonged incubation with a high concentration of HVJ (8,000 HAU ml^{-1}), did result in the release of LDH.

These observations indicate that histamine release from mast cells is directly induced by virus and that this release is similar to antigen-, dextran- and concanavalin A-induced reactions^{3,8,9}. These reactions are dependent on Ca^{2+} and temperature, and are potentiated by PS. It is therefore suggested that a common process, which is related to membrane function leading to exocytosis by fusion of the plasma and granule membrane, is involved in the mechanism of their histamine release.

It is well known that HVJ possesses haemolytic activity, but histamine release from mast cells by this virus could not be caused by its lytic action. This point is clear from the fact that HVJ-induced histamine release from mast cells was not accompanied by the leakage of LDH. Moreover, Ca^{2+} required in this histamine release acts in an inhibitory manner on the haemolytic activity of HVJ, and such haemolysis requires phosphatidylethanolamine; PS is not effective¹⁰. Recently, Homma *et al.*¹¹ have prepared HVJ that possesses cell fusion full activity and infectivity, but no haemolytic activity. A comparative study of the histamine release from mast cells is now being carried out using non-haemolytic and haemolytic preparations.

The mechanism of histamine release by HVJ and enhancement by PS, in the presence of Ca^{2+} still remains unclarified. The study, by immuno-freeze-etching, of the fusion of erythrocytes by HVJ has elucidated that when viral envelopes fuse with red blood cell membranes, agglutination of intermembranous glycoproteins occurs¹². Chi *et al.*¹³ have demonstrated that a displacement of intramembranous particles occurs at an early stage in the secretory reaction of mast cells. From these findings it seems that even at an early stage of infection of mast cells by HVJ there probably occurs a perturbation of the membrane structure, followed by clustering of intermembranous particles. It has been suggested that fusion of granule membrane and cell membrane takes place at the region of perturbed lipid bilayer denuded of intermembranous particles¹⁴. Ca^{2+} and PS in all probability, play a part in the enhancement of the formation of fluid cluster in the membrane, or the perturbation of lipid bilayer¹⁵. If PS is absent, the perturbation in the membrane induced by HVJ would rapidly be stabilised (Fig. 1b). The fact that histamine release induced by HVJ is highly sensitive to temperature also seems to indicate that membrane fluidity is important in membrane fusion.

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KATSUMI SUGIYAMA

Department of Pharmacology,
Okayama University Medical School,
Shikata-cho 2-5-1E,
Okayama 700, Japan

Received 31 January; accepted 19 October 1977.

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Immediate early antigens in human cytomegalovirus infected cells

DURING a comparison of cytomegalovirus (CMV)-specific haemagglutinating and immunofluorescent antibody titres in human sera, 3 of 60 sera tested by indirect immunofluorescence gave homogenous nuclear staining in 100% of infected cells within 1 h after infection (p.i.). These antigens differ from EBNA-type antigens described recently by Geder¹ in CMV-infected cells 3 h p.i. in that anticomplement immunofluorescence² is not required for their detection. They differ from 'early antigens' (EA)³ in that they appear much sooner and are located uniquely in the nucleus. Finally, they differ from 'late antigens' (LA) for they appear in the absence of DNA synthesis. By analogy to antigens described for the pseudo-rabies system by Ben-Porat *et al.*⁴ and for herpes simplex virus type 1 by Beth *et al.*⁵, we term these antigens 'immediate early antigens' (IEA).

CMV-IEA were partially characterised by studying their appearance in cells treated with inhibitors of DNA, RNA and protein synthesis, as well as in cells non-permissive for human CMV replication. The titres of each serum in antibodies to IEA, EA and LA is given in Table 1. The reactivity of two of the three IEA-positive sera (M19 and ICM) was studied in various conditions and compared with that of an IEA-negative serum (AUD).

When human diploid cells (MRC-5) were infected with 1 PFU (plaque-forming unit) per cell of Mira strain CMV, diffuse nuclear fluorescence was detected 1 h later using a 1/60 dilution of M19 or ICM (Fig. 1a). The serum AUD (diluted 1/60, Fig. 1b) failed to detect any intracellular antigens until 15 h after infection. Appearance of antigens detectable by IEA-positive sera was not influenced by treatment of cells before or after infection with inhibitors of DNA or RNA synthesis. Neither cytosine arabinoside ($20 \mu\text{g ml}^{-1}$) nor iododeoxyuridine ($100 \mu\text{g ml}^{-1}$) added at the time of infection, nor phosphonoacetic acid ($50 \mu\text{g ml}^{-1}$) added 24 h before and maintained after infection affected IEA formation. Actinomycin D added at the time of infection at $6\text{--}20 \mu\text{g ml}^{-1}$ failed to inhibit antigen formation even when cells were pretreated with this drug ($10 \mu\text{g ml}^{-1}$) for as long as 15 h before infection. Cycloheximide when added 18 h before infection and maintained after infection blocked antigen formation; however, when cycloheximide was added only 1 h before infection and maintained in the culture medium after infection, antigen formation was not blocked.

IEA-positive sera detected antigens induced by two strains of CMV (Mira and K9V), but not by HSV-1 (KOS and A 44 strains), HSV-2, or by a bat herpesvirus (Eidolon)⁷ when tested 3 h after infection at a dose of approximately 1 PFU per cell. Neutralisation of Mira strain CMV with either MIC or ICM at 37 °C for 1 h eliminated nuclear fluorescence. Incubation of virus at 37 °C for 1 h in growth medium or with a CMV-negative serum did not affect appearance of IEA fluorescence.

Ultraviolet light irradiation (30 min at 85 erg mm⁻² s⁻¹) and heat inactivation (30 min at 56 °C) of infecting virus

Table 1 Immunofluorescence titres of sera used to study immediate early (IEA) and late (LA) antigens in human cytomegalovirus infected cells

Serum	IEA*	CMF specific antigens	
		LA†	IgM†
M19	320	320	80
MIC	20	80	ND
ICM	80	320	0
AUD	0	320	ND

Human diploid cells (MRC-5) were grown in Leibovitz L-15 medium supplemented with 10% calf serum and infected with 1 PFU per cell of human cytomegalovirus by plating on to coverslips 0.2 ml of virus and 2 × 10⁶ cells in 0.2 ml in each well of 25-well Sterilin culture plates. Immunofluorescence was carried out 3 h p.i. when seeking IEA and 72 h p.i. when estimating LA and CMV-specific IgM antibody activity. All cells were washed in three changes of phosphate-buffered saline (PBS) containing Mg²⁺ and Ca²⁺ ions and fixed in two changes of ice cold methanol for a total of 5 min. Coverslips were air-dried, incubated with appropriate dilutions of test sera for 30 min at 37 °C when testing for IEA and LA, and for 3 h at 37 °C when testing for IgM. Coverslips were then washed in at least four changes of PBS over a period of 10 min and incubated with either FITC-conjugated goat-anti-human IgG (Melory) or with FITC-conjugated goat-anti-human IgM (Melory) for 30 min at 37 °C. After extensive washing in PBS, coverslips were fixed in 10% formol, washed with PBS and mounted in glycerine/PBS (9:1) mixture.

*Titres expressed as reciprocal of the highest dilution giving whole nuclear fluorescence.

†Titres expressed as reciprocal of the highest dilution giving ++ (maximum +++ fluorescence of nuclear inclusion bodies).

prevented IEA formation. Ultraviolet irradiation of cells for 10 or 20 min before infection did not inhibit antigen appearance. Nuclear fluorescence varied as a function of virus dilution; a 1/5 dilution gave 70% fluorescence and a 1/10 gave 20% fluorescence.

Human CMV induced IEA in non-permissive cells. Mouse fibroblasts (MLF-CBA, isolated at the Pasteur Institute) and epithelial cells (TCC₃₆, Pasteur Institute) reacted positively with IEA-positive sera 18 and 24 h, respectively, after infection with Mira strain CMV. IEA-negative serum revealed no antigens in these cells at any of the times studied. Rabbit ear skin fibroblasts (RSF, Pasteur Institute) showed 100% nuclear fluorescence 9 h after infection with either Mira or K9V CMV (Fig. 1c, d). In these cells, IEA-positive fluorescence disappeared on ultraviolet irradiation of infecting virus. None of the sera used in this study reacted positively with CMV transformed hamster fibroblasts (Cx90 3B) at passage 39.

If iododeoxyuridine (IUDR) 100 µg ml⁻¹ applied at the time of infection was used to block DNA synthesis, IEA disappeared from nuclei and appeared in the cytoplasm 45 h after infection, at which site they were barely detectable 72-h p.i. This would suggest that in the absence of DNA synthesis, IEA either disappear or lose their original antigenic structure after leaving the nucleus. IEA are probably not structural proteins of the virus since they are practically undetectable in cells blocked by IUDR 72 h after infection at a time when IEA-negative LA-positive serum detects antigens in both nucleus and cytoplasm. IEA might be related to CMV-induced stimulation of cellular protein synthesis known to occur early after infection⁸.

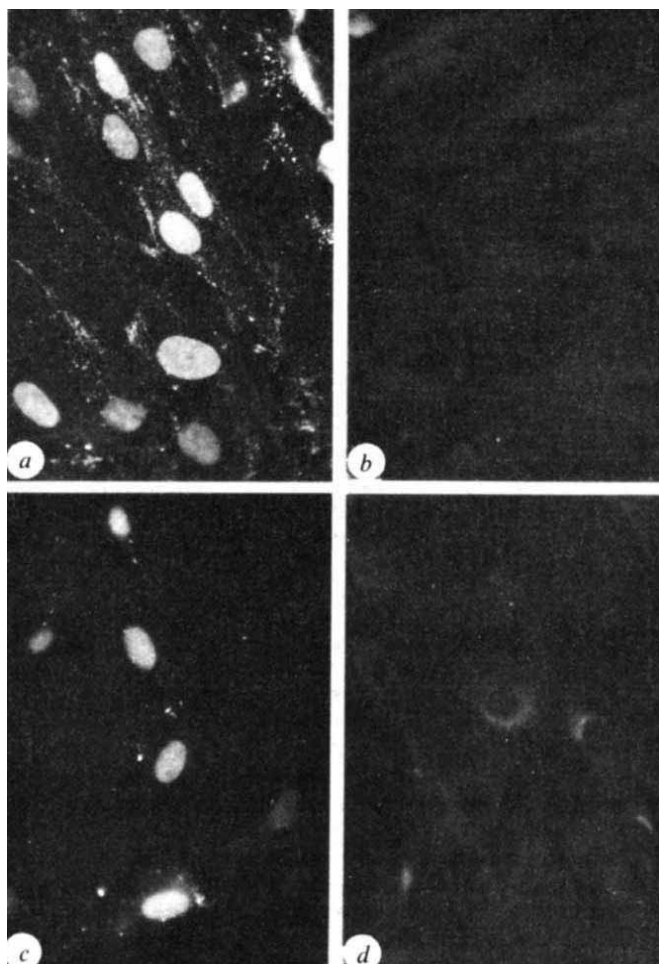
The significance of these antigens in the CMV replicative cycle remains obscure for three reasons. First, the abundance of these antigens in the nucleus within such a short time p.i. when viral DNA synthesis has not occurred. Second, these antigens appear in spite of inhibition of DNA and DNA-dependent RNA synthesis. And third, ultraviolet inactivation experiments show that functional virus DNA is required. The possibility that these antigens represent accumulation of infecting viral capsids in the nucleus seems to be ruled out since IEA-negative LA-positive serum gives no nuclear fluorescence and as electron microscope examination of cells 3 h after infection failed to show any viral capsids in cell nuclei.

IEA antibodies are rarely detectable in CMV positive sera. Among the donors of the three IEA-positive sera, there seems to be no common clinical background which would permit extrapolating from the patient's histories a coherent explanation for the relationship of these antibodies to the stage of disease.

Further characterisation of IEA must await polyacrylamide gel analysis and the production of specific antisera in order to characterise immunoprecipitates. This work is in progress.

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Fig. 1 Immunofluorescence of HCMV infected cells. *a*, Human lung fibroblasts (MRC-5)-IEA positive serum (M19 1/60) 3 h after infection. *b*, Same cells IEA-negative serum (AUD 1/60). *c*, Rabbit skin fibroblasts 18 h p.i.-IEA-positive serum (M19 1/60). *d*, Same rabbits cells-IEA-negative serum (AUD 1/60). (× 1,000).



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SUSAN MICHELSON-FISKE

Collège de France,
U. 112 of the Institut National de la Santé
et de la Recherche Médicale,
Laboratoire de Médecine Expérimentale,
75231 Paris, Cedex 05

FLORIAN HORODNICEANU

JEAN-CLAUDE GUILLON

Département de Virologie,
Institut Pasteur,
75724 Paris, Cedex 15

Received 14 June; accepted 25 October 1977.

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A single gene determines the host range of influenza virus

THE genome of influenza virus consists of eight distinct segments of single stranded RNA as determined by electrophoresis in polyacrylamide gels¹⁻³. Each of these segments codes for one of the eight virus specified polypeptides⁴⁻⁹ and is therefore believed to be a single gene which functions by a monocistronic messenger RNA¹⁰. Because different strains of influenza may vary in base sequence homology⁹ or in the electrophoretic mobilities of their RNA segments and infected cell polypeptides^{11,12}, it is often possible to determine the parental origin of every gene in an interstrain recombinant. Such procedures have been used to investigate the physiological properties associated with some of the influenza virus specified proteins^{9,13,14}. I describe here the application of this approach to an investigation into the genetic control of host range. The results show that the difference in host range between two avian influenza viruses depends solely on the properties of one of the internal viral proteins associated with RNA polymerase activity.

One of the major areas of interest in the study of influenza viruses is the genetic control of biological properties such as virulence¹⁵, pathogenicity¹⁶, plaque morphology¹⁷ and host range¹⁸. The gene coding for the major surface glycoprotein, the haemagglutinin, may be important in some of these properties. Proteolytic cleavage of the haemagglutinin is necessary for activation of progeny virions^{19,20} and is therefore required for multicycle growth in tissue culture and perhaps in the host animal. Since the degree of cleavage of the haemagglutinin varies between different influenza strains and in different cell types^{21,22}, Klenk¹⁹ has suggested that cell proteases may play an important part in the host range and spread of infection of these viruses. The pathogenicity of influenza viruses however, seems to be influenced by additional factors. Rott *et al.*¹⁶ have shown that the pathogenicity of fowl plague virus (an avian influenza A virus) is not exclusively dependent on the haemagglutinin nor on the other surface glycoprotein, the neuraminidase, and have suggested that an optimal combination of genes is necessary. Of considerable importance in determining whether a virus has the capacity to become a virulent pathogen would be a gene controlling its host range. Although the above already recognised factors are of undoubted importance, the presence of the correct host range gene would be an essential prerequisite for the emergence of a new strain virulent for a given host. This paper demonstrates that a single gene in influenza can effectively control host range as measured by plaque formation in tissue culture monolayers.

Two avian influenza A viruses (fowl plague virus; FPV) strains Rostock and Dobson were used. Both strains form clear plaques on chick embryo fibroblasts (CEF) but only FPV Dobson forms plaques on the mammalian derived BHK cells. Differences in the ability of influenza strains to form plaques is not uncommon, for example, A/WSN strain forms plaques in MDCK cells and CEF, whereas A/PR/8/34 strain forms plaques in MDCK cells²³ but not CEF²⁴. This phenomenon in many cases, however, has previously been attributed to the properties of the viral haemagglutinin and its degree of cleavage in the different cell types. In fact, plaque formation *in vitro* can be induced artificially in some cases by the addition of a proteolytic enzyme, such as trypsin, to the overlay medium²⁴.

The ability of FPV Dobson to form plaques in BHK cell monolayers did not depend on the properties of the haemag-

Table 1 Genotypes and plaque assay titres of FPV Rostock and FPV Dobson parents and recombinants

Virus	P ₁	P ₂ and P ₃	Parental origin of proteins					Plaque titres		
			HA	NP	NA	M	NS	CEF	BHK	
Rostock	R	R ²	R ³	R	R	R	R	R	9.5 × 10 ⁷	0
Dobson	D	D ²	D ³	D	D	D	D	D	1.4 × 10 ⁹	8.0 × 10 ⁸
Recombinants										
Di47c-1	R	R ²	R ³	R	R	R	R	D	7.5 × 10 ⁷	0
DiUS4-5	R	R ²	R ³	D	R	R	?	R	1.8 × 10 ⁸	0
DiUS1c-2	R	R ²	R ³	R	D	R	?	R	1.3 × 10 ⁸	0
Di39c-5	D	R ²	R ³	R	D	R	?	R	1.9 × 10 ⁸	0
DiMn5c-1	D	D ³	R ³	R	R	R	?	R	2.6 × 10 ⁸	1.4 × 10 ⁸
DiMn5c-3	R	D ³	R ³	R	D	R	?	R	1.9 × 10 ⁸	1.5 × 10 ⁸
Di39c-7	?	R ²	R ³	R	D	R	?	D	1.2 × 10 ⁸	0
Di44c-4	D	D ³	R ³	R	D	R	R	R	9.0 × 10 ⁷	1.0 × 10 ⁸
Dd45-n	R	D ²	R ²	D	D	?	?	R	4.3 × 10 ⁷	0
Di44-2	R	D ³	R ³	R	D	R	?	D	2.9 × 10 ⁸	1.6 × 10 ⁸
Di44c-1	R	D ³	R ³	D	D	R	?	D	3.3 × 10 ⁷	5.5 × 10 ⁷
DiMn5c-6	D	D ³	R ³	D	D	R	?	D	4.5 × 10 ⁸	2.3 × 10 ⁸
R47i-2	D	D ²	R ²	D	D	R	?	D	1.1 × 10 ⁷	0
Dd45-3	D	D ²	R ²	D	D	?	?	D	6.0 × 10 ⁷	0
Di44-7	D	D ³	R ³	R	D	D	D	D	2.3 × 10 ⁷	1.9 × 10 ⁷
Dd45-e	D	D ²	R ²	D	D	R	D	D	2.4 × 10 ⁷	0

The parental origin of proteins was determined for each recombinant by examining the electrophoretic mobilities of their infected cell polypeptides¹⁴, and their RNA segments of known coding function^{8,11}. P_{1,2,3}, Polymerase associated proteins; HA, haemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix protein; NS, non-structural protein 1; 0, no detectable plaques at any dilution. D², Dobson P₂; D³, Dobson P₃; R², Rostock P₂; R³, Rostock P₃.

glutinin but on the presence of a gene coding for one of the polymerase associated proteins, P₃. The method used involved the construction of recombinants between FPV Dobson and FPV Rostock and the determination of the parental origin of each gene or gene product in these recombinants, as has been described previously¹¹. Several such hybrids have been tested for their ability to form plaques in BHK cell and CEF monolayers. The plaque titres obtained, together with the genotype of each recombinant are shown in Table 1. In Table 1 the polymerase-associated proteins P₁, P₂ and P₃ are numbered purely on the order of their electrophoretic mobilities in polyacrylamide gels^{4,10}, and, because mobilities of proteins vary between different influenza virus strains^{11,12}, it is not necessarily valid to assume that the three P proteins will migrate in the same order in all strains. Extensive recombination studies in this laboratory have shown that the P₂ of Rostock and the P₃ of Dobson are functionally equivalent. Therefore any recombinant involving these two proteins will have either Rostock P₂ and Dobson P₂ or Rostock P₃ and Dobson P₃. In Table 1, therefore, D³ and R³ refer to the Dobson and Rostock P₃ proteins respectively, and D² and R² to the P₂ proteins. All the recombinants that form plaques in BHK cell monolayers have the Dobson P₃ protein (D³): this is the only viral gene product common to all the BHK plaque-forming recombinants. Furthermore the haemagglutinin (HA) is not involved in this control since recombinants which possess the Dobson P₃ protein form plaques on BHK cells irrespective of the origin of the HA (for example, DiMn5c-1, and Di44c-1) and recombinants which lack this protein but still

possess the Dobson HA, are unable to form plaques. Also pulse chase experiments of infected cell polypeptides (Fig. 1) suggest that the HA of FPV Rostock is cleaved in BHK cells even though it cannot form plaques.

These results strongly suggest that the gene coding for the P₃ protein of Dobson confers the ability to undergo multicycle growth in BHK cell cultures. Further experiments are necessary to establish the exact function of this protein in the infectious cycle but the results of Palese *et al.*¹³ suggest that it may be important in the synthesis of virion RNA. The fact that the equivalent protein, P₂ of FPV Rostock, although synthesised, does not function correctly in these mammalian derived cells, may imply a specific host factor interaction with this protein.

It is possible that mutations in other viral genes will be shown to affect selectively multiplication in particular cell types and that the above observations merely reflect the host range limitation of the FPV Rostock strain. On the other hand, the viral gene product recognised here may be a common host range controlling factor in all influenza viruses. If this is so, the study of its mechanism of action should lead to a better understanding of host-virus interactions which occur in influenza virus multiplication^{25,27}, and the gene coding for this protein (gene 1) would be worthy of special consideration in the construction of attenuated recombinant viruses for use as live vaccine in man.

J. W. ALMOND*

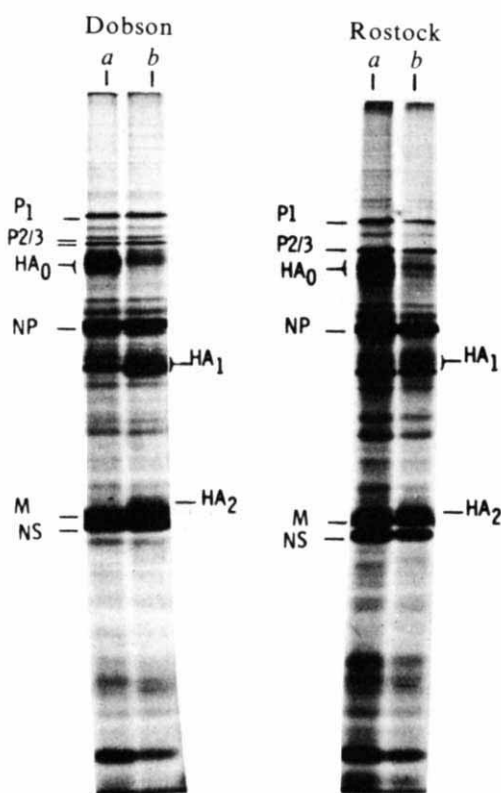
Division of Virology,
Department of Pathology,
University of Cambridge,
Laboratories Block, Addenbrooke's Hospital,
Hills Road, Cambridge, UK

Received 7 July; accepted 30 September 1977.

*Present address: Sandoz Forschungsinstitut, Brunner Strasse, 59 A1235 Vienna, Austria.

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Fig. 1 Pulse chase experiments: Tracks (a) show FPV Rostock and FPV Dobson infected cell polypeptides synthesised during a 15-min pulse with ³⁵S-methionine at 4 h after infection. b, Similar samples which have been 'chased' for 45 min by adding medium containing cold methionine after removal of the label. Samples were prepared and subjected to polyacrylamide gel electrophoresis as described by Inglis *et al.*⁴.



Foot-shock induced stress increases β -endorphin levels in blood but not brain

FOOT-SHOCK induced stress promotes a five to sixfold increase in β -endorphin plasma levels. Similar increases were also found for ACTH plasma levels. In the hypothalamus, foot-shock induced stress promotes a decrease of β -endorphin assayed by radioimmunoassays. These data suggest that physiological increases in plasma β -endorphin levels induced by stress do not result in elevated levels of brain β -endorphin.

Akil *et al.*¹ have shown that acute stress induced by inescapable

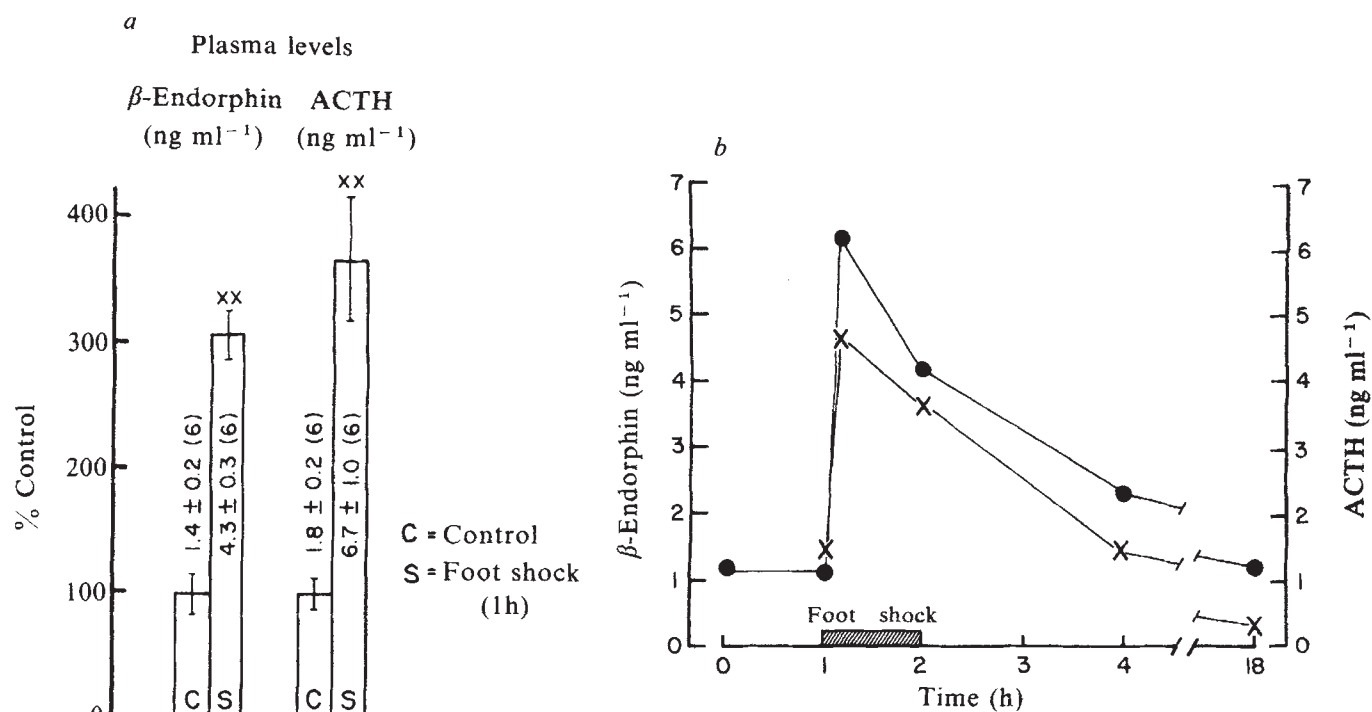


Fig. 1 a, Stress and plasma levels of ACTH and β -endorphin. Rats (Sprague Dawley, male 200 g) were stressed by exposure for 1 h to inescapable electric foot shocks (1 mA, 1 s duration, at random 12 per min). Directly after the end of the stress period, they were decapitated and the trunk blood was collected on EDTA. After immediate centrifugation, the plasma was frozen until RIA. Randomised controls from the same lot of animals were killed at the same time (10 a.m.) directly after their removal from their cages. Their plasma samples were processed together with the samples of the stressed animals. **b**, Time course study of blood levels for ACTH ($\times$) and β -endorphin ($\bullet$) after stress. A jugular cannula was implanted in the right jugular of a rat (Sprague Dawley, male 200 g). The cannula was flushed each day with a solution of heparin. Three days after the surgery, the stress experiment as described in Fig. 1a was performed. The rat was placed in the experimental Perspex box one hour before the first electric foot shock. Blood samples were drawn 1 h and 1 min before the first shock and at the subsequent times indicated and were processed as described above.

electric foot shock promotes analgesia in rats and that this analgesic effect of stress was partially reversed by naloxone, a morphine antagonist. Simultaneously with that report, the explosion of observations began which characterised the endogenous brain peptides with opioid activity enkephalins^{2,3} and endorphins⁴⁻⁶. Therefore, it was tempting to seek correlations between the stress-induced analgesia and the levels of the brain opioid peptides. Madden *et al.*⁷ reported that levels of opioid brain materials were increased after stress. That study, however, used radio-receptor displacement assays which can not distinguish among the several endogenous peptides which share this biological property *in vitro*. Therefore, it was revealing when Fratta *et al.*⁸ using a radioimmunoassay for Met⁵-enkephalin, were unable to detect any increase in brain immunoreactive material after stress. However, they proposed that another opioid peptide, β -endorphin, could have been mobilised from pituitary by stress to accumulate in brain tissue.

We have observed here that which β -endorphin is mobilised from the pituitary during stress and accumulates up to sixfold in plasma, the released β -endorphin does not accumulate in brain. On the contrary, hypothalamic levels of β -endorphin are slightly, but significantly reduced after the same 30 min episode of electric foot-shock stress.

Double antibody radioimmunoassay (RIA) for β -endorphin was previously described^{9,10}. The RIA is specific to the Leu¹⁴-His²⁷ segment¹⁰. As β -endorphin is the C-terminal 31 amino acid fragment of β -lipotropin, this RIA could reflect any β -lipotropin in our samples. The 31,000 MW pre-prohormone isolated from clonal cultures of mouse adenohypophyseal tumour cells can also be read in this β -endorphin RIA¹¹. Nevertheless, gel filtration sizing experiments have shown that the β -endorphin immunoreactivity in our samples (plasma and hypothalamus extracts) was associated mainly with a compound eluting in a pattern non-distinguishable from the one of synthetic β -endorphin¹². ACTH RIA was performed using a procedure previously described¹³.

Two series of rats (Sprague Dawley, male, 200 g) were stressed by inescapable electric foot shock (1 mA, 1 s duration, at random 12 shocks per min) during 30 min or 1 h. Such treatment clearly produces stress as shown by a more than three-fold increase in both β -endorphin and ACTH plasma concentration (Fig. 1a). The peak of the stress-induced secretion was found 10 min (Fig.

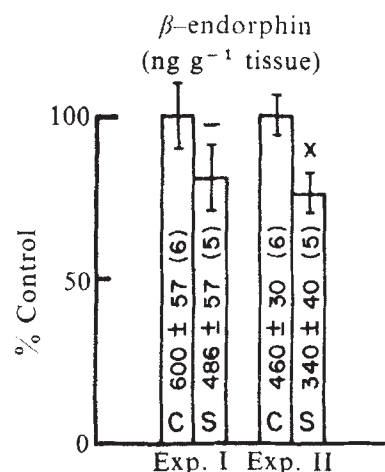


Fig. 2 Hypothalamus content in opioid peptides after acute stress. Two stress protocols were used. In experiment I (Expt I) animals were stressed exactly as in the protocol described in Fig. 1a. In experiment II (Expt II) the foot shocks were given for only 30 min and after a delay of 15 min after the shocks the animals were killed. In both experiments, brains were quickly removed and dissected as outlined by Glowinski & Iversen¹⁹. The tissues were frozen on dry ice, weighed and poured in 2 ml of 1 M acetic acid preheated to 98 °C. After 15 min in the boiling bath, the tissues were homogenised (polytron, setting 6, 10 s). After centrifugation (1,000g for 1 h) the supernatant was neutralised with 1 M NaOH and frozen before RIA. Results are expressed in ng per g of tissue.

1b) after the start of the foot shocks for both ACTH and β -endorphin which are secreted concomitantly in equimolar amounts, as previously reported¹⁴. Brain levels of β -endorphin were measured after 1 h of stress. No significant differences were observed in any brain region except in hypothalamus where the concentration of β -endorphin immunoreactivity was decreased. This difference, however, was not statistically significant. The stress experiment was then repeated, using a protocol identical to that of Akil *et al.*¹ consisting of foot shock for 30 min followed by a delay of 15 min before killing. With this protocol, again, a decrease in β -endorphin immunoreactivity was observed only in hypothalamus, and this difference was now statistically significant. No difference was observed in the other parts of the brain, pituitary, or pineal.

Stress or acupuncture stimulation are reported to induce analgesia which is in part reversed by naloxone^{1,15,16}. Our results here indicate that while the analgesia may be concurrent with an increase of the secretion of β -endorphin from the pituitary into blood, the brain content of β -endorphin shows decreased content in hypothalamus and no change elsewhere. This decrease could most simply reflect the net effect of the release and degradation of the β -endorphin peptide from hypothalamic nerve processes. Indeed β -endorphin is stored like a neurotransmitter in neurones (ref. 12 and to be published). Nevertheless, we cannot with confidence correlate our findings with the mechanisms involved in pain suppression. Although intravenous infusion of β -endorphin induces analgesia in mice at doses of 8 mg kg⁻¹ or more^{17,18}, we find no analgesia in rats after even higher doses. Also, the blood concentrations reached by such doses would be more than three orders of magnitude higher than the sixfold changes observed after the physiological shifts with stress observed here. Further work is required to establish whether endocrine secretion of pituitary β -endorphin and ACTH are causally related to the central analgesia produced by stress or whether hypothalamic β -endorphin content is depleted as a result of the behavioural disturbances evoked by our catastrophic stimuli. The present observations suggest, however, that increased blood levels of β -endorphin are not reflected by increases in brain β -endorphin.

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JEAN ROSSIER
EDWARD D. FRENCH
CATHERINE RIVIER
NICHOLAS LING
ROGER GUILLEMIN
FLOYD E. BLOOM

The Salk Institute for Biological Studies,
La Jolla, California 92037

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Naloxone alters pain perception and somatosensory evoked potentials in normal subjects

THE discovery of opiate-like peptides (endorphins) in brain, blood and cerebrospinal fluid^{1,4} raises questions as to the physiological role of these substances. Given the prominent analgesic effects of opiates, the regulation of pain perception is a candidate for endorphin action. Endorphins microinjected into the periaqueductal grey matter of rats induce analgesia which is reversed by naloxone, a pure narcotic antagonist⁵. If endorphins play an active part in the regulation of pain, then naloxone administered to man should alter pain appreciation. Naloxone has not been shown to have analgesic, respiratory, euphoric, pupillary or electroencephalogram (EEG) effects in man⁶. Studies in rats^{7,8} and one early study in man⁹ have suggested slight hyperalgesic effects with naloxone. El-Sobky *et al.*¹⁰ failed to demonstrate naloxone effects on electric shock pain judgments in five subjects. In the experiments reported here, subjects were divided into pain sensitive and pain insensitive subgroups. The insensitive subjects found shocks significantly more painful after naloxone administration while the sensitive group experienced them as less painful. Evoked potentials showed similar significant group differences. These results suggest that individual differences in pain sensitivity may relate to differences in an endorphin system.

Normal adult volunteers (10 male, 11 female; mean age 20 yr) gave informed consent and participated in three similar experimental sessions. Each session consisted of two psychophysiological pain rating tests and a pain somatosensory evoked potential (EP) test. The initial session was for familiarisation and no drug was given. On the following two sessions the subjects either received an intravenous injection of 2.0 mg naloxone (0.4 mg ml⁻¹) or an equal volume of saline (0.9%) in a random order and double-blind fashion. Experimental procedures began 5 min after injection and lasted 20 min. All procedures involved single shocks administered to the left forearm by a computer-controlled constant current stimulator with a Tursky electrode¹¹. Subjects received three shocks at each milliamperage increment from 1 to 31 mA for a total of 93 shocks in a random sequence at 2.5-s intervals. Subjects rated each shock in one of four categories; noticeable, distinct, unpleasant or very unpleasant. Signal detection analysis yielded two pain measures: a nonparametric analogue of response criterion and a sensitivity level¹². The sensitivity measure has been associated with pharmacological analgesia¹⁴ and response criterion related to suggestion effects¹⁵.

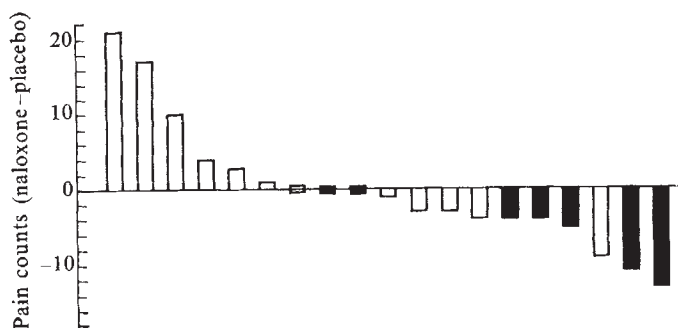


Fig. 1 Pain counts (unpleasant plus very unpleasant subjective ratings) illustrated as the difference between naloxone and placebo days for each individual studied. Positive differences thus indicate more stimuli rated as unpleasant or worse on naloxone than on placebo. Subjects were divided into pain-insensitive and pain-sensitive groups on the basis of a sensitivity measure derived from signal detection analysis during a separate pre-drug trial session. Note that no pain-sensitive subject became more sensitive on naloxone while most pain-insensitive individuals did (groups significantly different by *t* test on individual difference scores $P < 0.05$). Two subjects were excluded because no stimulus was rated above distinct on the familiarisation day and thus the sensitivity could not be calculated. Open bars, pain-insensitive subjects; solid bars, pain-sensitive subjects.

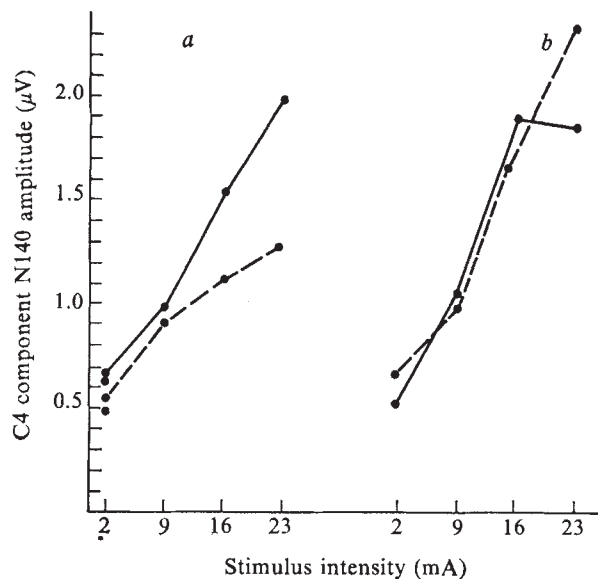


Fig. 2 Mean evoked potential amplitude in μV for negative peak at about 140 ms at four stimulus intensities for pain sensitive and insensitive subject groups. Note EP differences at highest stimulus intensities. Data were analysed using a three-way analysis of variance with repeated measures for the intensity and drug dimensions and independent groups for the third dimension; linear trend analysis was used on the intensity dimension. Group differences were statistically confirmed (group $\times$ drug $\times$ intensity interaction, $F = 4.45$, 1, 17 d.f. $P < 0.05$). EP differences between sensitive and insensitive groups on placebo can also be seen. *a*, Pain-insensitive group. *b*, Pain-sensitive group. Solid lines, naloxone; broken lines, placebo treatment.

In addition, we computed the mean amperage associated with each stimulus category and we counted the number of responses out of the total of 93 judged unpleasant and very unpleasant (termed 'pain counts'). The evoked potential procedure used four stimulus levels (2, 9, 16, and 23 mA) presented 64 times at 1-s intervals in a random order; EEG was recorded from vertex and somatosensory cortex (C4) and averaged separately for each intensity¹³. Evoked potential (EP) amplitude was measured for the three major EP components, a positive P100 (76–112 ms), a negative N140 (116–152 ms) and a positive P200 (168–248 ms)¹³.

Subjects were divided into pain sensitive and pain insensitive groups based on whether their pain sensitivity level on the familiarisation day fell above or below the mean of subjects used in two previous pain studies^{12,13}. Individuals so defined as pain insensitive showed naloxone hyperalgesia. They rated stimuli of lower milliamperage as unpleasant and judged a higher number of the 93 stimuli as unpleasant after naloxone administration, whereas pain sensitive individuals showed the opposite effect (Fig. 1). Familiarisation session sensitivity significantly predicted 'pain count' changes between placebo and naloxone ($r = 0.49$, $P < 0.05$). While both the sensitivity and the response criterion measures similarly indicated greater naloxone hyperalgesia in the pain insensitive group, neither measure reached statistical significance. But, the distinct/unpleasant response criterion determined for the familiarisation session did predict the change by naloxone in the mean milliamperage for the very unpleasant category ($r = -0.47$, $P < 0.05$).

Similar group differences were seen for EP results (Fig. 2). Peak N140 amplitude for the vertex lead increased following naloxone by $0.36 \mu\text{V}$ in the insensitive group but decreased in the sensitive group (group by drug interaction, $F = 4.47$, 1, 17 d.f. $P < 0.05$). In EPs recorded from C4, this effect was especially pronounced at the highest shock intensities. EP latency was also significantly shorter after naloxone in the insensitive group especially at low intensities for P100, N140 and P200 (analysis of variance on each of three peaks, $P < 0.05$, group by intensity by drug interaction). The sensitive group showed increases in latency at low intensity

for all three peaks. The groups as divided on the pain sensitivity measure also differed in the rate of increase in EP amplitude with increasing stimulus intensity of the C4 P100 component: pain insensitive individuals showing lower amplitude/intensity slopes ($F = 4.27$, linear trend effect $P < 0.05$). This pattern has been referred to as 'reducing' and previously reported in other pain insensitive groups¹⁶.

When subjects were examined as a single group (Fig. 3) the P100 latency for the lowest intensities was significantly decreased following naloxone. The N140 component of the EP also demonstrated shorter latency for all subjects after naloxone ($F = 5.17$, $P < 0.05$) with no effect in P200 latency. Neither sensitivity nor response criterion measures for the distinct/unpleasant division were significantly different between drug conditions. Naloxone administration also failed to change the mean EP amplitude across intensities or the amplitude/intensity slope for P100, N140 and P200.

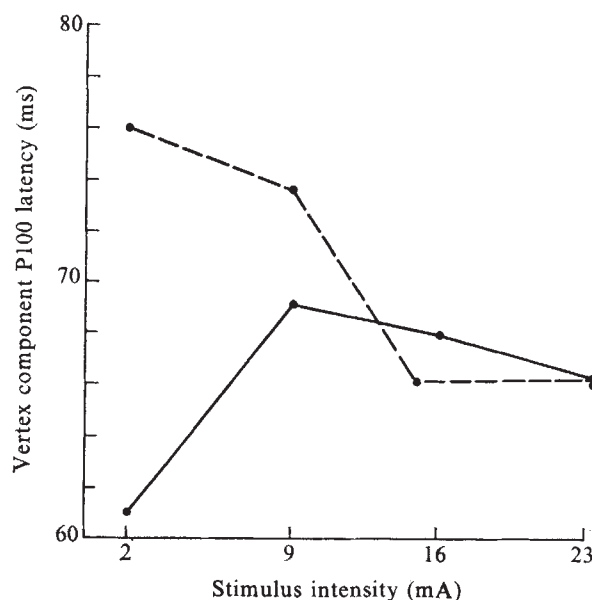


Fig. 3 Mean evoked potential latency in ms for the positive component at about 100-ms post-stimulus for naloxone and placebo conditions for all 21 subjects. This effect was statistically confirmed by 2-way ANOVA, ($F = 6.41$, drug by intensity interaction, 1, 20 d.f., linear trend effect, $P < 0.05$). Solid line, naloxone; broken line, placebo treatment.

Our finding of naloxone effects on pain response supports the hypothesis that the endogenous opiate-like substances have a physiological role in pain regulation. The finding of group differences both in EPs and in subjective ratings is not merely a statistical artefact as a totally independent session was used to categorise the subjects, and as both groups changed it does not seem to reflect only response ceiling or floor effects. Individuals with relatively higher levels of endorphins, relatively more opiate receptors or relatively more highly developed pain inhibitory pathways might be more naloxone responsive. If these factors were the only source of individual variation, however, one might have expected to see only the pain insensitive group respond to naloxone, rather than the bidirectional effects observed. Lasagna⁹ noted a "strange biphasic quality" of naloxone response with 2 mg apparently causing analgesia but higher doses having hyperalgesic effects. Similarly supporting bidirectionality, Leybin *et al.*¹⁷ found an unexpected increase in the perception of pain in rats given the endogenous opiate Met-enkephalin. Bidirectional effects may reflect individual differences in the functional activity of complementary pain modulation systems, rather than a dose dependent effect at receptor sites. A primary analgesic action of endogenous opiate-like substances similar to the actions of

exogenous opiates is not clearly supported by our data. The bidirectional effects observed here and elsewhere suggest a modulatory rather than strictly analgesic role for endorphins.

MONTE S. BUCHSBAUM
GLENN C. DAVIS
WILLIAM E. BUNNEY, JR

Unit on Perceptual and Cognitive Studies,
National Institute of Mental Health,
9000 Rockville Pike,
Bethesda, Maryland 20014

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Do snail neurones contain more than one neurotransmitter?

THE 5-hydroxytryptamine (5-HT) present in the giant neurone in each metacerebral ganglion (GSC) of the snail, *Helix pomatia*, is probably a transmitter^{1–3}. The neurone also contains acetylcholine⁴, a putative transmitter and its synthesising enzyme, choline acetyltransferase (Ch.Ac., EC 2.3.1.6) (ref. 5). These observations, together with a report showing multiple putative transmitter substances in certain *Aplysia* neurones⁶, have resulted in several challenges^{2,6,7} of the validity of Dale's hypothesis that each neurone contains and releases only one transmitter. The GSC is found in other gastropods, in every case containing 5-HT (refs 9,10); there is high Ch.Ac. activity in *Helix*⁵, but in *Aplysia* the enzyme is absent¹¹ or present in trace amounts⁸. All previous work on *Helix* neurones has been carried out on hand-dissected GSCs, and so contamination from glial and other small neurones must always be considered (see ref. 12). If a neurone is considered to be spherical, a slight increase in the radius would result in a substantial volume of substance being trapped between the neuronal membrane and the contaminating membrane. The isolation process is thus of critical importance. I have reanalysed the neurone for a spectrum of transmitters to investigate whether the GSCs can synthesise 'common' transmitter substances. A neurone-designated cell 21^{2,5,13}, situated on the posterior-dorsal surface of the visceral ganglia and known to contain high levels of Ch.Ac. was also examined for comparison. I show that the GSC contains only 5-HT, with no significant Ch.Ac., and that it also contains trace amounts of dopamine and histamine.

Single neurones were dissected from fresh snails (*Helix pomatia*) caught locally and the isolated perikarya were freed from adhering tissue with special care¹². Four neurones of each type were placed in 10- μ l capillary tubes containing snail physiological saline¹⁴ and then washed thoroughly by centrifugation and removal of the supernatant. This process was repeated and the neurones analysed for their histamine¹⁵, octopamine¹⁶, dopamine and noradrenaline¹⁷, Ch.Ac. (ref. 18), 5-HT (refs 12, 19, 20) and amino acid content^{12,19}.

A second series of experiments tested the ability of the two neurone types to synthesise transmitters. Circumoesophageal ganglia were incubated in 1 ml of snail saline¹⁴ with 30 μ M of previously purified ³H-precursors (all with specific activities of 12–23 Ci mmol⁻¹) to transmitters for 60 min at 26 °C and individual cells dissected¹². Groups of four cells were washed twice with physiological saline and homogenised in their capillary tubes in 50% acetone containing 0.1 N HCl. After centrifugation,

the supernatants were analysed by two-dimensional chromatography on silica gel plates for amine transmitters (see Fig. 1) or by one-dimensional chromatography on cellulose plates for acetylcholine (see Table 2).

The results show clearly (Table 1) that the only putative transmitter present in unique and high concentrations in the GSC is 5-HT, thus differing from the report of Hanley *et al.*⁵ that the neurone also contains high amounts of Ch.Ac. The assay conditions for Ch.Ac. might not have been optimal, but this was taken into consideration. Moreover, the activity of Ch.Ac. found in cell 21 is of the same order as that found by Hanley *et al.*⁵. The GSCs also contain trace amounts of histamine and dopamine, as does cell 21, which also has a minute amount of 5-HT. Since it is impossible to dissect an absolutely 'clean' neurone because of glial investment, adhering satellite cells and exogenous substances released from other damaged cells¹², and since highly sensitive assay procedures are used, the background noise due to impurities

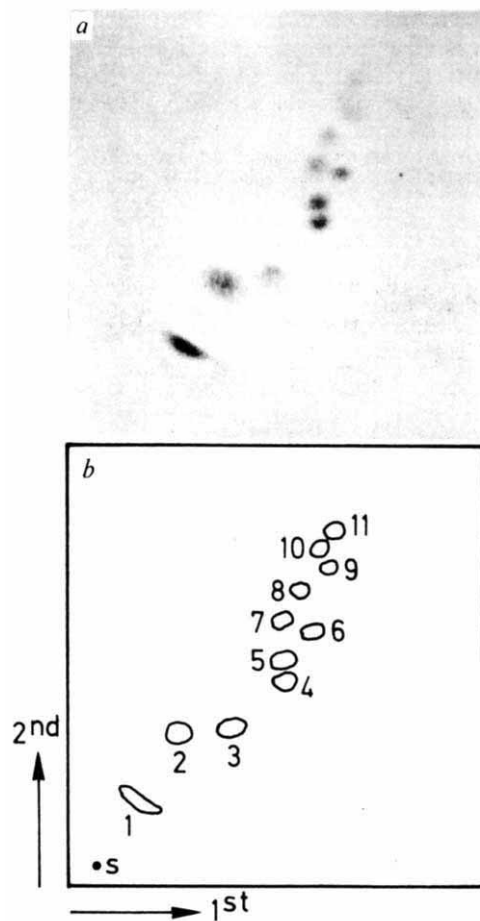


Fig. 1 a, A chromatogram (precoated silica gel 5 × 5 cm) and b, a corresponding map to show the relative positions of various transmitter substances and precursors separated in two solvent systems. The directions of chromatography are indicated by the arrows and the solvent systems used were butanol–acetic acid–water (15:3:5 by vol.) in the first direction and butanol–pyridine–acetic acid–water (15:2:3:5 by vol.) in the second direction. The substances were visualised on the chromatograms by initial exposure to paraformaldehyde vapour for 30 min at 80 °C followed by spraying with 1% potassium ferricyanide in ammonium hydroxide solution. After drying, the substances were sprayed with ninhydrin solution. S, Starting point; 1, glutamate; 2, GABA; 3, DOPA; 4, 5-hydroxytryptophan; 5, tyrosine; 6, tryptophan; 7, noradrenaline; 8, dopamine; 9, 5-hydroxytryptamine; 10, octopamine; 11, tyramine. Individual spots were then removed from the chromatogram, the substance was eluted with methanol and the amount calculated by scintillation spectrometry and, assuming the specific activity of the products to be equal to the ³H-precursor, added to the ganglia at the start of the incubation. Recovery of all tritiated substances was in the range of 60–80%. Cell 21 did not metabolise ³H-tyrosine, ³H-tryptophan or ³H-tyramine but the GSCs formed ³H-5-hydroxytryptophan and ³H-5-HT from the ³H-tryptophan. The possibility of the formation of ³H-GABA from glutamate was not tested, since whole brain tissue cannot form GABA.

Table 1 Transmitter candidates in GSCs and cell 21 of *Helix pomatia*

Substance	Concentration in the GSC	Concentration in cell 21	Procedure used (ref.) and sensitivity actually achieved
5-Hydroxytryptamine	3.8×10^{-4} M	2×10^{-8} M	ref. 20 500 pg and dansyl method ¹⁹ 100 pg
Histamine	2×10^{-8} M	10^{-8} M	ref. 15 100 pg
Octopamine	N.D.	N.D.	ref. 16 50 pg
Dopamine	$8-10^{-9}$ M	3×10^{-8} M	ref. 17 50 pg
Noradrenaline	N.D.	N.D.	ref. 17 50 pg
Ch.Ac.	0.2 pmol per cell per h	61 pmol per cell per h	ref. 18 Blank corresponded to 1% of radioactivity in incubation medium
Glutamate	6×10^{-4} M	3×10^{-4} M	Dansyl method ¹⁹ 100 pg
Aspartate	7×10^{-5} M	9×10^{-5} M	
Glycine	5×10^{-4} M	5.9×10^{-4} M	
Taurine	6×10^{-6} M	6×10^{-6} M	

The total volume of each cell type was estimated by measuring the diameters of a number of cells (mean diameter of both cell types 120–140 μ m) under light microscopy. It was found that the GSC and cell 21 had volumes of 1.2 nl. The molarity (results reported for 5–7 determinations; in each experiment four neurones were pooled) of each substance in the two neurone types could then be calculated.

N.D. = not detected.

will tend to be amplified. It is therefore suggested that the trace amounts of histamine, dopamine and Ch.Ac. in the GSC are artefacts. Interesting in the original publication of Emson *et al.*¹³ is a note describing the activity of Ch.Ac. in the GSCs as 'small', whereas in the later publication⁵ the Ch.Ac. activity in the GSC is of the order of 21 pmol per cell per h. The concentration of the putative transmitter amino acids in both cell types was similar, suggesting that they do not have a transmitter function in these neurones.

incubation solution had no effect, substantiating previous data¹.

These results suggest that the GSCs produce and contain one putative transmitter substance, 5-HT, and not ACh. Cell 21 seems, in contrast, to use only ACh. The question of whether multiple neurotransmitters exist in neurones^{4–8} should therefore be reassessed critically, especially when the evidence is biochemical and drawn from hand-dissected neurones, where the degree of error may be magnified. Trace quantities of substances could be endogenous in origin, since from the evolutionary point of view,

Table 2 ^3H -ACh and ^3H -5-HT formed from 30 μ M ^3H -choline or ^3H -tryptophan

	^3H -ACh, fmol		^3H -5-HT, fmol	
	Without eserine	With eserine	Without pargyline	With pargyline
per GSC	$0.2 \pm 0.2^\dagger$ (12)	$0.5 \pm 0.2^\dagger$ (12)	9 ± 0.9 (5)	9 ± 1 (5)
per cell 21	$18 \pm 3^\dagger$ (12)	$56 \pm 6^\dagger$ (12)	$0.2 \pm 0.2^*$ (5)	$0.2 \pm 0.2^*$ (5)
per cerebral ganglion	$3,000 \pm 105$ (3)	$4,900 \pm 195$ (3)	71 ± 4 (3)	69 ± 5 (3)

All values are $\pm$ s.e.m. with the number of determinations (in each determination four neurones were pooled) in parentheses, and were calculated by taking into consideration the specific activity of the ^3H -choline and ^3H -tryptophan. ^3H -Amine neurotransmitters were fractionated from other substances by two-dimensional chromatography on 5×5 -cm precoated silica gel plates (see Fig. 1) while ^3H -ACh was separated from ^3H -choline by single-dimensional chromatography on 5×5 -cm precoated cellulose plates using the solvent system butanol-ethanol-acetic acid-water (8:2:1:3 by vol.). ^3H -ACh was visualised by iodine vapour and eluted from the chromatogram with methanol, and the absolute amount of ^3H -ACh calculated by assuming the specific activity to be equal to ^3H -choline added to the ganglia at the start of the incubation. Recovery of ^3H -choline and ^3H -acetylcholine was 85%.

*Not significantly different from blank values (Student's *t* test $P > 0.05$).

† Significantly different from experiments without eserine and with blank values (Student's *t* test $P < 0.05$).

The results of the second series of experiments supported the first. Initial experiments showed that snail ganglia can synthesise ^3H -ACh, ^3H -5-HT, ^3H -dopamine and ^3H -octopamine from their precursors, but not ^3H -GABA or ^3H -noradrenaline, thus confirming previous results³. The amount of ^3H -ACh formed was approximately 50-fold that of any other ^3H -transmitter. Cerebral ganglia contained ~ 3 pmol of ^3H -ACh per ganglia after incubation with 30 μ M ^3H -choline (Table 2), but only 0.07 pmol of ^3H -5-HT, 0.06 pmol ^3H -dopamine or 0.03 pmol ^3H -octopamine following incubation with 30 μ M ^3H -tryptophan, ^3H -tyrosine or ^3H -tyramine. The amount of ^3H -ACh formed was further increased when incubated with 10^{-4} M eserine (see Table 2). This study showed that small amounts of ^3H -transmitters could be detected in specific neurones, especially if the cells produced ACh. In fact, ^3H -ACh was readily detected in extracts of four isolated cell 21 neurones removed from ganglia previously incubated with ^3H -choline (Table 2). In contrast, only a trace amount of ^3H -ACh was found in extracts of either four or eight pooled GSCs. Since the limit of resolution of these experiments was of the order of 0.3 fmol of ^3H -ACh, about 2% of the level in a single cell 21, the inability to detect ^3H -ACh in the GSCs is probably not due to a lack of precursor, but to the absence of the synthetic enzyme. Inclusion of eserine in the incubations (Table 2) produced a very small but statistically detectable quantity of ^3H -ACh in the GSCs, and the amount of ^3H -ACh in the cell 21 was increased by a factor of 3.

The only other transmitter synthesised by the neurones was ^3H -5-HT which was limited to the GSCs, producing about 9 fmol ^3H -5-HT. A monoamine oxidase inhibitor (pargyline 10^{-4} M) in the

all neurones are expected to contain the same genetic machinery. It will then remain to demonstrate that they are of physiological significance⁸ and ask why neurones, which use the same transmitter substance, nevertheless, metabolise 100-fold different concentrations of the substance.

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NEVILLE N. OSBORNE

Max-Planck Institut für
experimentelle Medizin,
Forschungsstelle Neurochemie,
3400 Göttingen, FRG

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α adrenoreceptors but not β adrenoreceptors increase in rabbit uterus with oestrogen

STEROID hormones alter sympathetic function and modulate responses to catecholamines¹⁻³. The gonadal steroids, oestrogen and progesterone, can affect sympathetic response by altering catecholamine metabolism, and, in addition, they produce qualitative changes in the contractile response of smooth muscle to stimulation by adrenaline and noradrenaline⁴⁻⁶. The response of human oviduct and uteri from several species changes from contraction to relaxation depending upon the concentrations of gonadal steroids. Contraction, which is mediated by α -adrenergic receptors, is observed in uteri from oestrogen-treated humans or rabbits, while relaxation, a β -adrenergic response, predominates during pregnancy or with progesterone treatment. Conversion between α and β response, depending on hormonal or other environmental factors, has prompted the hypothesis that α - and β -adrenergic receptors may be interconvertible⁷⁻⁹. We used the radioligands ³H-dihydroergocryptine (DHE) and ¹²⁵I-iodohydroxybenzylpindolol ([¹²⁵I]IHYP) to quantitate α - and β -adrenergic receptors respectively in subcellular preparations of uteri from rabbits treated with oestrogen or oestrogen followed by progesterone. α -Adrenergic binding sites were three times greater in uteri from the oestrogen-treated animals in which α -adrenergic response was predominant than in uteri from animals treated with oestrogen followed by progesterone in which β -adrenergic response predominates. The number of β -adrenergic binding sites was unchanged by the different treatments and these sites were only 5% as numerous as α -adrenergic sites. These findings suggest that gonadal steroids may alter smooth muscle adrenergic response by alterations of adrenergic receptor number and do not support the hypothesis that α - and β -adrenergic receptors are simply interconvertible.

After injecting immature New Zealand rabbits intramuscularly with 150 μ g estradiol benzoate for 4 d, we prepared myometrial strips and examined isometric muscle contractility *in vitro*. Such strips exhibit spontaneous contractile activity, and contractions are increased in force and

frequency when 5×10^{-6} M noradrenaline is added. The stimulatory effect is inhibited by the α -adrenergic antagonist phentolamine (10^{-5} M). When the 4 d of oestrogen treatment is followed by 4 d of treatment intramuscularly with 10 mg d⁻¹ progesterone, 5×10^{-6} M noradrenaline reduces spontaneous myometrial activity. This inhibitory effect is blocked by the β -adrenergic antagonist propranolol (10^{-5} M).

We prepared a subcellular fraction of myometrium from the animals after these treatment courses and studied binding of radioligands for the α - and β -adrenergic receptors under identical conditions. Dissociation constants (K_d) and numbers of binding sites were determined in equilibrium binding experiments (Table 1). Data were normalised to DNA content to correct for hyperplasia and hypertrophy of the myometrium.

α -Adrenergic receptors were quantitated with the radioligand DHE. Williams *et al.*¹¹ have shown, and we have confirmed, that binding of this agent is compatible with interaction at an α -adrenergic receptor site in rabbit myometrium. β -Adrenergic receptors were quantitated with the radioligand [¹²⁵I]IHYP. [¹²⁵I]IHYP, a potent β -adrenergic antagonist, binds to subcellular preparations of several cells in a manner compatible with interactions at the β -adrenergic receptor¹²⁻¹⁶. In subcellular preparations of rabbit myometrium the binding of [¹²⁵I]IHYP shows high affinity ($K_d = 2 \times 10^{-10}$ M), low capacity (71 fmol mg⁻¹ DNA), and is readily reversible ($t_{1/2} = 5$ min). The binding is inhibited by β -adrenergic agonists and antagonists, the active (–)-isomers being 20 times more potent than the corresponding (+)-isomers. The rank order of potency of adrenergic agonists for competition with [¹²⁵I]IHYP is isoproterenol > epinephrine > norepinephrine—identical to the order for inhibition of spontaneous uterine activity by these agents⁵.

Subcellular preparations of myometrium from uteri of oestrogen-treated rabbits in which α -adrenergic response was predominant had three times as many DHE binding sites as did uteri in which β -adrenergic response was predominant (progesterone treatment). This change in the number of binding sites was not accompanied by a significant change in apparent affinity of the binding sites for the radioligand (Fig. 1). Control animals, which were un-

Table 1 Dissociation constants and number of binding sites for DHE and [¹²⁵I]IHYP in oestrogen- or progesterone-treated rabbits

	DHE binding		[¹²⁵ I]IHYP binding	
	Dissociation constant (nmol l ⁻¹)	Number of binding sites (fmol mg ⁻¹ DNA)	Dissociation constant (nmol l ⁻¹)	Number of binding sites (fmol mg ⁻¹ DNA)
Oestrogen	5.1 ± 1.1	3,400 ± 400	0.21 ± 0.03	86 ± 11
Progesterone	8.0 ± 2.8†	1,000 ± 200*	0.22 ± 0.02†	56 ± 9†

* $P < 0.001$.

† $P > 0.05$.

Uteri were removed, scraped free of endometrium and disrupted with a Vertis homogeniser. The crude homogenate was filtered through cheesecloth and then centrifuged at 600 and 1,000g for 15 min each. The pellets were discarded after each centrifugation and the resulting supernatant was centrifuged at 30,000g for 15 min. The pellet was resuspended in *N*-2 hydroxyethylpiperazine-*N'*-2-ethane sulphonic acid (HEPES), pH 7.5, 4 mM MgSO₄, 1 mM dithiothreitol (DTT) and used for [¹²⁵I]IHYP and DHE binding assays. 0.1 ml aliquots of the suspension containing 1.8 mg ml⁻¹ protein ([¹²⁵I]IHYP assay) or 4.4 mg ml⁻¹ protein (DHE assay) were added to 0.1 ml HEPES 50 mM, pH 7.5, 4 mM MgSO₄, 1 mM DTT containing increasing concentrations of radioligand (0.1 to 20 times K_d) and 0.02 ml of 1 mM HCl or 10 μ M (–)-propranolol ([¹²⁵I]IHYP assay) or 10 μ M phentolamine (DHE assay) in 1 mM HCl. Incubations were at 30 °C for 15 min (DHE) or 60 min ([¹²⁵I]IHYP assay). Binding was terminated in the [¹²⁵I]IHYP assay by adding to the incubation solution 1.5 ml of 0.1 mM (±)-propranolol (1 mM MgSO₄, 20 mM K₂HPO₄, pH 7.5 (wash buffer) and immediately filtering the sample through Gelman A-E glass fibre filters. The filters were washed for 30 s with 30 ml of wash buffer at 37 °C. In the DHE assay the incubation was terminated by adding 5 ml of 5 mM HEPES, pH 7.5 and 2 mM MgSO₄ at 23 °C and immediate filtering through Whatman GF/C filters. The filters were then washed with 15 ml of the same solution at 23 °C. Specific binding is defined as the difference between binding in the absence and presence of 10 μ M unlabelled antagonist (propranolol in the [¹²⁵I]IHYP assay and phentolamine in the DHE assay). Data were analysed by a nonlinear iterative curve fitting program and normalised to sites per mg DNA¹⁰. Results are means of individual site number and K_d determinations from 5 experiments ± s.e.m. Statistical analysis was by Student's unpaired *t* test.

Parallel experiments were carried out in which the filtrate and wash were collected and refiltered after DHE or [¹²⁵I]IHYP binding and the usual filtration. The refiltration was done three times. More than 90% of the specifically bound radioactivity of both ligands was present on the first filter.

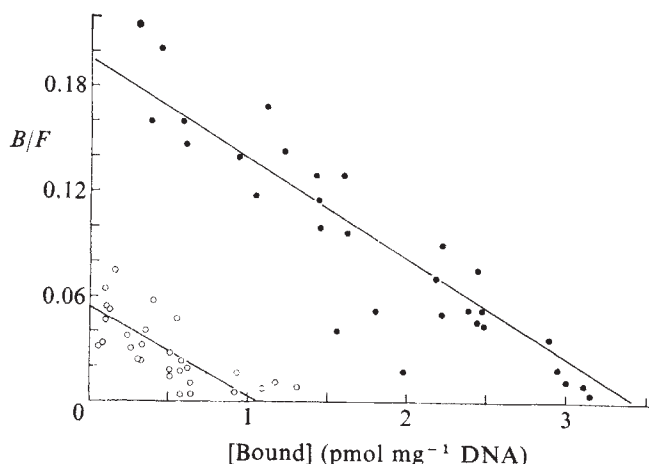


Fig. 1 Scatchard analysis of DHE binding experiments with particulates from oestrogen-treated (●) or oestrogen followed by progesterone-treated (○) rabbits. Data is normalised to binding per mg DNA and points are means of duplicate determinations in five experiments with each treatment.

treated or injected with vehicle instead of progesterone, had numbers of DHE binding sites similar to progesterone-treated animals, indicating that the sites were increased by oestrogen. Quantitation of [125 I] IHYP binding sites demonstrated that β -adrenergic predominance in uteri from progesterone-treated animals could not be explained by an increase in [125 I] IHYP binding sites or by altered affinity of the receptor for [125 I] IHYP. Indeed, there was a small but not statistically significant ($0.1 > P > 0.05$) increase in [125 I]-IHYP binding sites in oestrogen-treated uteri (in which α -adrenergic response predominates) (Table 1).

DHE binding sites were 10–40 times more numerous than [125 I] IHYP binding sites. The difference in the number of α - and β -adrenergic binding sites could not be attributed to the filtration procedure used to determine radioactivity bound to the myometrial preparation (Table 1). Quantitation of β -adrenergic binding sites using the lower specific activity radioligand 3 H-dihydroalprenolol also resulted in approximately the same number of binding sites as did [125 I] IHYP. Binding studies of the initial homogenate had high levels of nonspecific binding but demonstrated similar changes in α -adrenergic binding sites with the different treatments and confirmed the relative differences in numbers of α - and β -adrenergic sites. In experiments in which we used NaF-stimulated adenylate cyclase to monitor membrane recovery, we found that the differences between the number of α - and β -adrenergic sites could not be attributed to differences in membrane recovery.

The smaller number of myometrial α -adrenergic binding sites after progesterone treatment in the rabbit accompanies the conversion of a contractile to an inhibitory response, and this could be a result of the change in the number of binding sites. Whether the change in the ratio of α - to β -adrenergic receptor sites from 15:1 to 50:1 in the presence of oestrogen is by itself sufficient to change myometrial response to catecholamines from inhibitory to contractile cannot presently be determined. Because oestrogen and progesterone, however, have additional effects on myometrial metabolism and function^{17–19}, it is possible that the alteration in contractile response results from the interaction of several events. The adrenergic component of other changes observed during pregnancy or treatment with oral contraceptives including cardiac output, vascular reactivity and metabolism, may also be influenced by such altered response^{20–24}. Recent studies in liver and heart have demonstrated alterations in adrenergic receptor numbers in response to glucocorticoids and thyroid hormone respectively^{1, 25, 26}.

It has been postulated that α - and β -adrenergic receptors are structurally related and may in fact represent alternate configurations of the same active site^{7–9}. The changes in numbers of binding sites in oestrogen- and progesterone-treated animals cannot be explained by a conversion of β - to α -adrenergic receptors. The number of β -binding sites was about 5% of the number of α -binding sites and was not changed with the addition of progesterone, while α sites decreased during progesterone treatment by 70%. If the decrease in α -receptor number was attributable to a conversion of α to β receptors, up to a 40-fold increase in β receptors would have been anticipated. This direct estimate of α - and β -adrenergic receptors does not support the hypothesis that the receptors are simply alternate configurations of the same gene product(s), and is more compatible with the concept that they are separate entities.

These studies suggest an important role for the regulation of adrenergic receptors by hormones acting at the level of the cell nucleus, and complement studies which have demonstrated regulation of adrenergic receptors by adrenergic agonists^{27–29}.

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JAMES M. ROBERTS
PAUL A. INSEL
ROBERT D. GOLDFIEN
ALAN GOLDFIEN

Department of Obstetrics, Gynecology and
Reproductive Sciences, Medicine and the
Cardiovascular Research Institute,
University of California, San Francisco,
San Francisco, California 94143

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Killing of single neurones by intracellular injection of proteolytic enzymes

To approach problems of sprouting, regeneration, and specificity of neuronal connections, it would be useful if a technique were available for selectively destroying an individual neurone or group of neurones without damaging other cells. One established method for eliminating a class of cells is to use the genetic approach and produce mutants. This, however, is often impractical for animals with long generation times. In invertebrate preparations, surgical removal of the cell body is not sufficient, since neuronal processes can survive and continue to function normally for weeks or months after they have been severed from their cell bodies^{1,2}. We have used the central nervous system of the leech to establish a technique for destroying individual neurones one at a time. The leech is a convenient preparation since the nerve cells are large, easily recognisable, and many have been identified and their synaptic connections and peripheral fields are well known^{3,4}. We have found that Pronase, a mixture of proteolytic enzymes (Calbiochem Pronase, free of nucleases, B grade), can be injected by pressure into an identified cell. With time, it spreads throughout the neurone's central and peripheral processes and destroys them.

The experiments were made with microelectrodes filled with 0.5% Pronase, 2% dye (fast green) and 50 mM KCl.

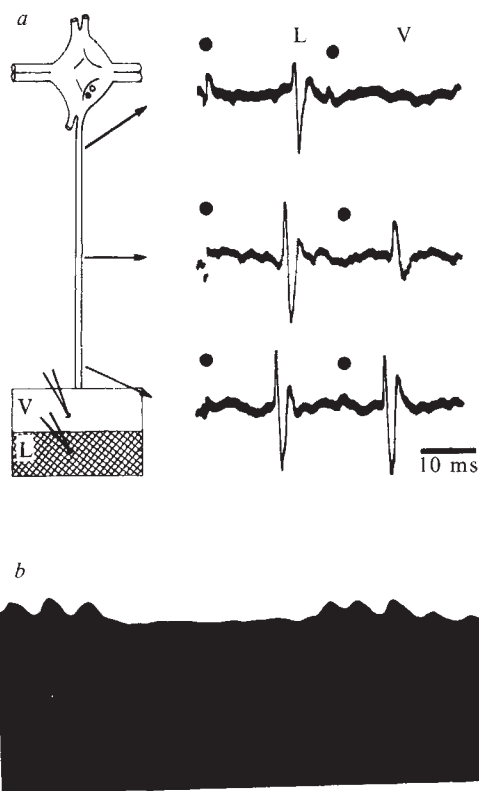


Fig. 1 *a*, Impulses recorded extracellularly from the axons of two sensory cells innervating lateral (L) and ventral skin (V) (ref. 3). One cell (V) had been injected with Pronase 18 h beforehand when its receptive field and that of L were mapped. In the experiment, impulses initiated by touching ventral skin failed to propagate into the ganglion while those from lateral skin continued to do so. The dots above the records indicate the instant at which the skin was indented by the piezo electric crystal. *b*, Photographic silhouette taken of part of a leech in which two motor neurones (in adjacent ganglia) responsible for reflex erection of the skin into ridges were injected with Pronase. The region of skin normally innervated by two cells (six annuli) lost the reflex.

The dye allowed visual monitoring of the injection and the KCl allowed the membrane potential to be recorded. The tips of the electrodes were bevelled to diameters between 1 and 2 μm and pressures of 1 to 5 pounds per square inch were applied. Injections usually took less than 5 s and the cell's resting and action potentials were still normal at the time the electrode was removed. Injections were made in isolated ganglia and in whole animal preparations. Isolated ganglia were cultured for periods of days after the injections⁵. Experiments using whole animals were carried out by anaesthetising them with 0.15% chlorobutanol and making a small incision through the ventral skin to expose a single ganglion. With oblique illumination it was possible to recognise individual cells and to confirm their identity by the characteristic shape of the action potentials⁶. The animals survived for many weeks at room temperature after the operations.

To follow the progress of cell destruction, a ganglion was removed still attached to the segment of skin it innervates. A mechanosensory cell that responds to light touch⁷ (touch cell) was injected with Pronase. Its sensory terminals were stimulated by a piezo electric crystal that indented the skin and extracellular recordings of the evoked action potentials were made from the nerve root connecting the skin to the ganglion. As a control, a neighbouring touch cell that innervates an adjacent area of skin was stimulated with a second crystal.

During the first 1 or 2 h after the injection, the cell body progressively lost its resting potential and ability to generate action potentials. Mechanical stimulation, however, of the skin still evoked action potentials travelling toward the ganglion that could be recorded from the roots. Over the next 20 h, conduction of action potentials along the axon failed at greater distances from the cell body (Fig. 1*a*) and by 48 h, the most distal process, millimeters from the site of the injection, was unresponsive. Conduction along the process of the control touch cell (Fig. 1*a*, L) which runs in the same nerve root, was not affected. In similar experiments in which a touch cell was injected in the whole animal, no recovery of function was observed over a 2-week period and the injected cell body could no longer be seen in the ganglion under a dissecting microscope. In control experiments cells that were injected with fast green and KCl alone showed none of these degenerative changes.

Functional evidence that the injected cells were destroyed was provided by injecting annulus erector motor neurones that are part of a reflex arc responsible for erection of the annuli into ridges⁴. Figure 1*b* is a photograph showing a silhouette of part of a leech in which a single motor neurone was injected in each of two neighbouring ganglia in a whole animal. Within 24 h the reflex was lost over the 6 annuli that are innervated exclusively from these neurones⁴. No recovery was apparent over a period of several weeks. Mechanical stimulation of the skin in dissected preparations from these animals continued to evoke normal sensory responses. When the ganglia were inspected, the motor neurones could not be seen but the ganglia appeared normal otherwise.

Anatomical evidence for the spread of Pronase through the cell's fine branches and into its terminals was provided by experiments in which neurones were injected with horseradish peroxidase (HRP) (ref. 6) and then with Pronase. Figure 2 shows an experiment in which two touch cells were injected with HRP. Four hours later, the cell on the right side of the ganglion was injected with Pronase, the cell on the left with a solution of KCl and dye without Pronase. Development of the cells for HRP staining 12 h later showed almost no reaction product in the cell body or central processes of the Pronase-injected cell. The reaction product, however, was dense in the cell's more distal processes in roots and connectives (out of the field) where the Pronase presumably had not yet reached, demonstrating

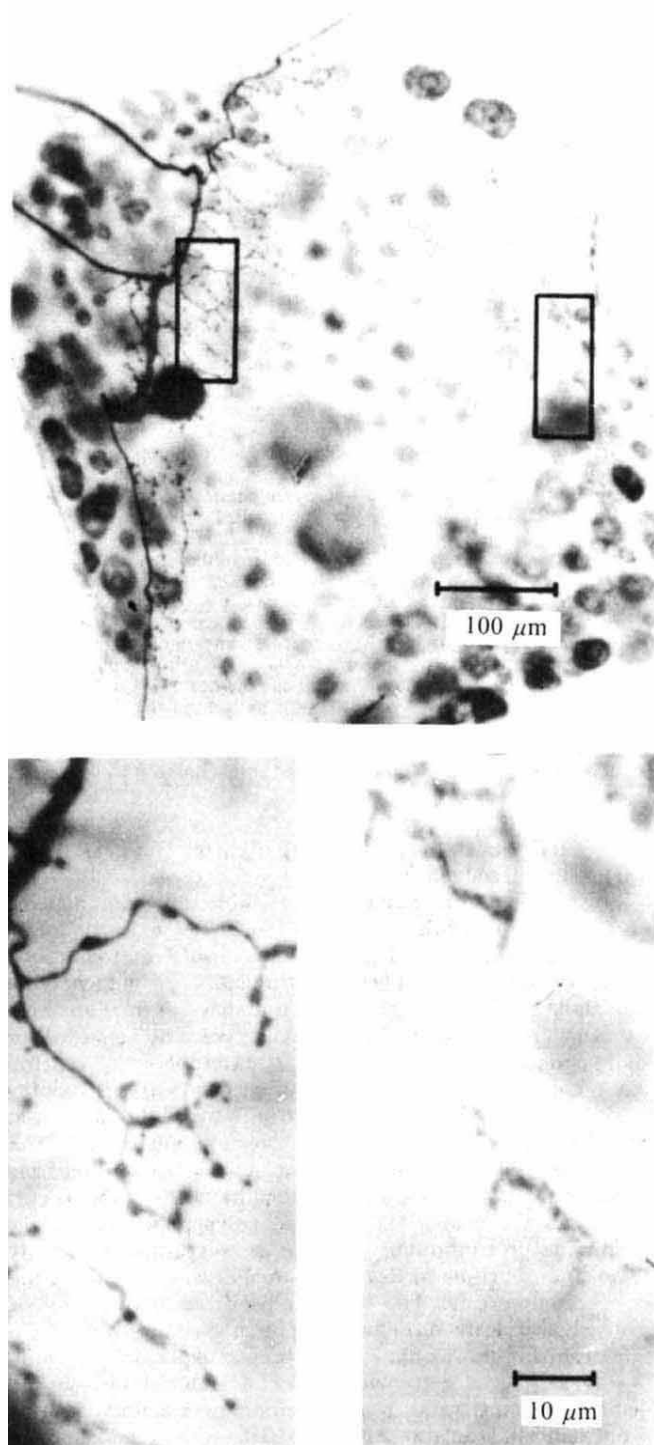


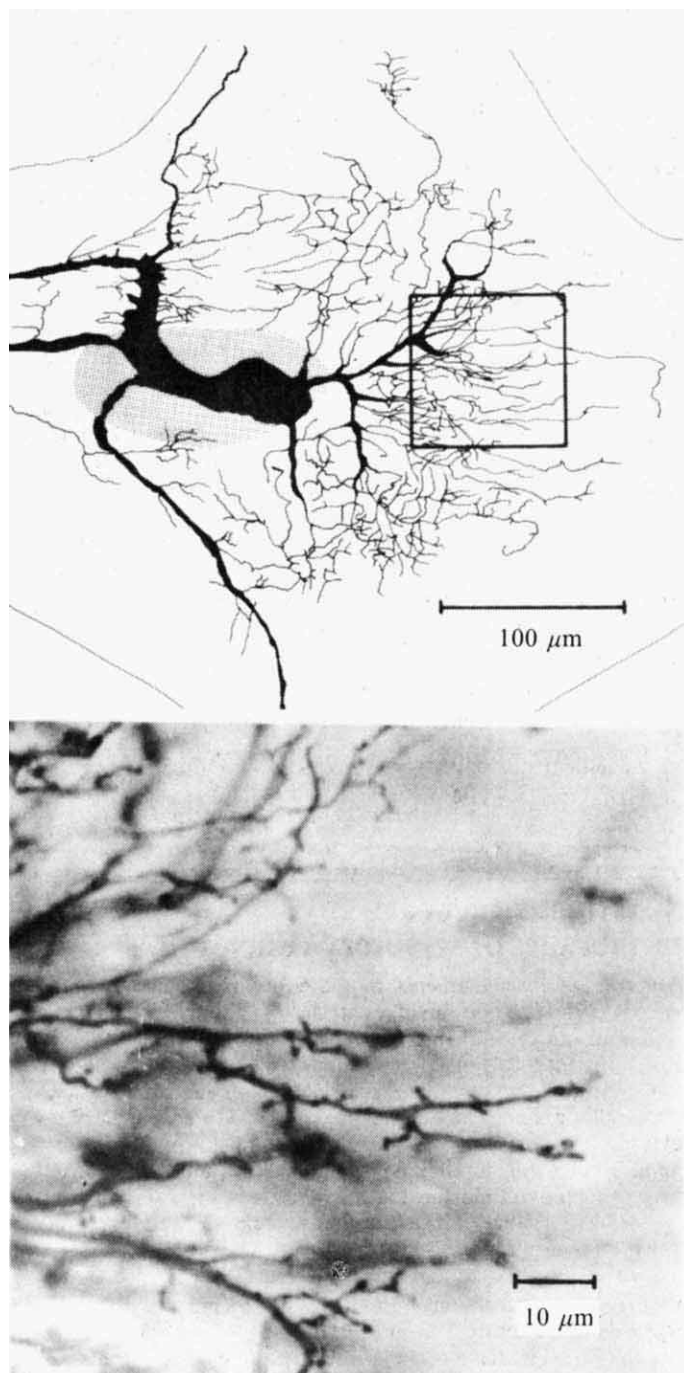
Fig. 2 Leech ganglion in which one touch cell on each side of the ganglion was injected with HRP. Four hours later, the touch cell on the right was injected with Pronase. After another 15 h, the peroxidase reaction was allowed to develop. The cell injected with Pronase showed only scattered processes within the neuropil but its axons could still be discerned at a distance from the cell body in roots and connectives, indicating that the HRP injection had been successful.

that the HRP injection had been successful. Processes of the control cell appeared normal.

It is important to establish that the direct effects of Pronase are confined to the injected cell. We have not been able to devise a quantitative assay for direct damage to other neurones, but a number of tests have failed to reveal any characteristic morphological or physiological changes in the properties of other cells. Injecting Pronase extra-

cellularly under the connective tissue capsule around the ganglion had no apparent effect on the electrical properties of the cells. Cells that are synaptically coupled to or in close proximity to an injected cell seemed to be anatomically and physiologically normal. For example, one of the two Retzius cells was injected with Pronase and two weeks later, the ganglion was removed from the animal and the remaining Retzius cell was injected with HRP (Fig. 3). Although the two Retzius cells are electrically coupled and form synapses in the same region of the neuropil, the fine

Fig. 3 Camera lucida drawing of a Retzius cell injected with HRP and a photograph of some of its fine processes in the neuropil. Two weeks earlier, the other Retzius cell in the ganglion had been injected with Pronase. The Pronase-injected Retzius cell had disappeared by this time but the arborisation of the cell injected with peroxidase (which had been coupled to the Pronase-injected cell) still appears normal. The stippled area represents the cell body.



processes of the remaining cell appeared to be undamaged and its physiological properties were unchanged. Results from this cell and other experiments showed that the synaptic potentials, action potentials, and input impedances of non-injected cells remained normal. No obvious changes were found even when a large number of other cells in the ganglion (up to a dozen) were injected with Pronase. The fine structure of non-injected cells also seemed normal. A particularly favourable cell to investigate by electron microscopy is the S neurone⁷. This cell sends a large axon through the middle connective (Faivre's nerve) that can readily be identified by its size and position. After an S cell was injected with Pronase, its degenerating axon could be seen in Faivre's nerve, but the surrounding axons and glial processes appeared normal (also K. Muller, personal communication).

Although these experiments do not rule out more subtle forms of damage to fine processes or to cell surfaces caused by Pronase leaking out of the injected cell, we conclude that injection of Pronase destroys all the processes of a single neurone without obvious damage to other cells. This technique may be particularly useful in simple invertebrate preparations, like the leech, in which one can now study the effects of removing one cell at a time instead of making less selective lesions that involve hundreds or even thousands of axons. For example, if a cell is killed, will sprouting occur in cells that are normally pre- or post-synaptic to it? Will processes grow to form new synapses replacing terminals removed by the Pronase injection? Even without obvious morphological changes, the efficacy of synaptic transmission between two cells might be altered by removal of a third neurone supplying input to the synapse. The technique may also be useful in determining functional roles of specific neurones and for analysing their connections to other cells.

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I. PARNAS*
D. BOWLING

Department of Neurobiology,
Stanford University Medical Center,
Stanford, California 94305

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*Present address: Neurobiology Unit, Hebrew University, Jerusalem, Israel.

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α -Actinin attached to membranes of secretory vesicles

THE role of microfilaments in secretion has been much debated^{1–4}. If secretion involves a sliding filament mechanism similar to muscle contraction⁵, microfilaments must attach at least transiently to vesicle membranes. Indeed, there is evidence that contractile proteins are associated with secretory vesicles (chromaffin granules) of adrenal medulla^{6,7}. Moreover, the outer (cytoplasmic) surface of secretory vesicles must provide 'anchoring molecules' for microfilaments. In skeletal muscle, the structural protein α -actinin located in the Z-line⁸ serves as such an anchoring molecule. An α -actinin-like protein was isolated from a variety of non-muscle cells^{9,10} and there is evidence that the same protein is attached to the inner (cytoplasmic) surface of the plasma membrane of non-muscle cells in association with microfilaments^{11–14}. Using a monospecific antibody to porcine α -actinin (Fig. 1) we have demonstrated the presence of this protein

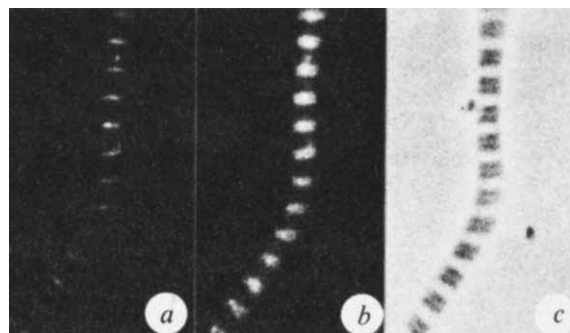


Fig. 1 Rat myofibril stained by means of indirect immunofluorescence. Antibody was raised in rabbits against purified native porcine skeletal muscle α -actinin¹⁵ precipitated with alum¹³. A crude IgG fraction was passed over an α -actinin Sepharose affinity column¹⁶ and the monospecific antibody was eluted at pH 2.7 (ref. 17). This antibody preparation gave a single precipitation line when tested in double diffusion against crude or purified preparations of the antigen. An antibody-Sepharose column retained only α -actinin from a muscle homogenate. The isolated myofibril (c) was incubated with this antibody followed by a, rhodamine-conjugated sheep antiserum against rabbit IgG (ref. 18); then b, incubated with human anti-actin serum¹⁹ followed by fluorescein-conjugated goat antiserum against human IgG (Miles Servac, Switzerland). The myofibril was then washed and mounted in 90% glycerol, 10% phosphate-buffered saline. The width of the rhodamine-positive bands and the distance between them (2.35 μ m) (a) as well as the comparison between a and b indicate that the antibody against α -actinin is fixed exclusively to the Z-lines. Preincubation of the anti- α -actinin antibody with purified α -actinin abolished only staining of the Z-lines.

in secretory vesicle membranes from chromaffin cells of the adrenal medulla and from platelets.

Bovine adrenal chromaffin granules were isolated in buffered sucrose with or without 0.15 M KCl (ref. 6). 5-Hydroxytryptamine-storing (5-HT) vesicles were isolated from rabbit blood platelets by gradient centrifugation²⁰; mitochondria and alpha granules²¹ were recovered in different fractions from the same gradient. Aliquots of the same gradient were used for sodium dodecyl sulphate (SDS) gel electrophoresis, electron microscopy and indirect immunofluorescence on frozen sections.

Membranes which were prepared from isolated chromaffin and 5-HT granules by hypotonic lysis contain polypeptides with molecular weights of α -actinin and actin (Fig. 2). Preparing chromaffin granules in low ionic strength buffers (without KCl) diminishes the proportion of those polypeptides among the membrane proteins (data not shown), suggesting a selective removal of α -actinin at low ionic strength with the actin component bound to it⁶. The actin-like band in the 5-HT granule profile is also clearly distinguishable but not as conspicuous as in the chromaffin granule pattern. The profiles of platelet mitochondria (Fig. 2c) and α -granule polypeptides (not shown) do not demonstrate any band at the position of α -actinin. A very prominent band can also be detected in the platelet mitochondria pattern at the position of actin. The proportion of this actin-like component among the total proteins varied considerably (by a factor of 2) with different preparations.

Indirect immunofluorescence was carried out on 4- μ m frozen sections of pelleted chromaffin granules, 5-HT granules, platelet mitochondria and α -granules. α -Actinin antibodies were fixed only to chromaffin granules from adrenal medulla and platelet 5-HT granules (Fig. 3c), whereas α -granules and platelet mitochondria (Fig. 3a) showed no fluorescence. All fractions were confirmed by electron microscopy to be pure homogeneous preparations. Furthermore, chromaffin granule membranes isolated in high ionic strength buffer (0.15 M KCl) and solubilised in 1% SDS showed a single line of precipitin reaction in a double-diffusion test against α -actinin antibody.

Parallel immunofluorescent staining with antibodies against actin¹⁹ was positive on several fractions from rabbit platelets, including α -granules and mitochondria. This result, together with the finding of a variable amount of an actin-like protein

among the mitochondrial polypeptides (Fig. 2) may reflect a contamination of platelet mitochondria with actin from the platelet cytoplasm, although by morphological criteria, these preparations were homogeneously pure (Fig. 3). In the adrenal medulla preparations immunofluorescent staining of different fractions with anti-actin was positive only on the chromaffin granules.

These data indicate that α -actinin is a constituent of the membrane of monoamine storage vesicles, but not of cellular organelles in general, and they add more weight to the hypothesis that α -actinin in plasma membranes and secretory vesicle membranes serves as an anchorage molecule for microfilaments.

We acknowledge the technical help of Miss K. Kelley. B.M.J.

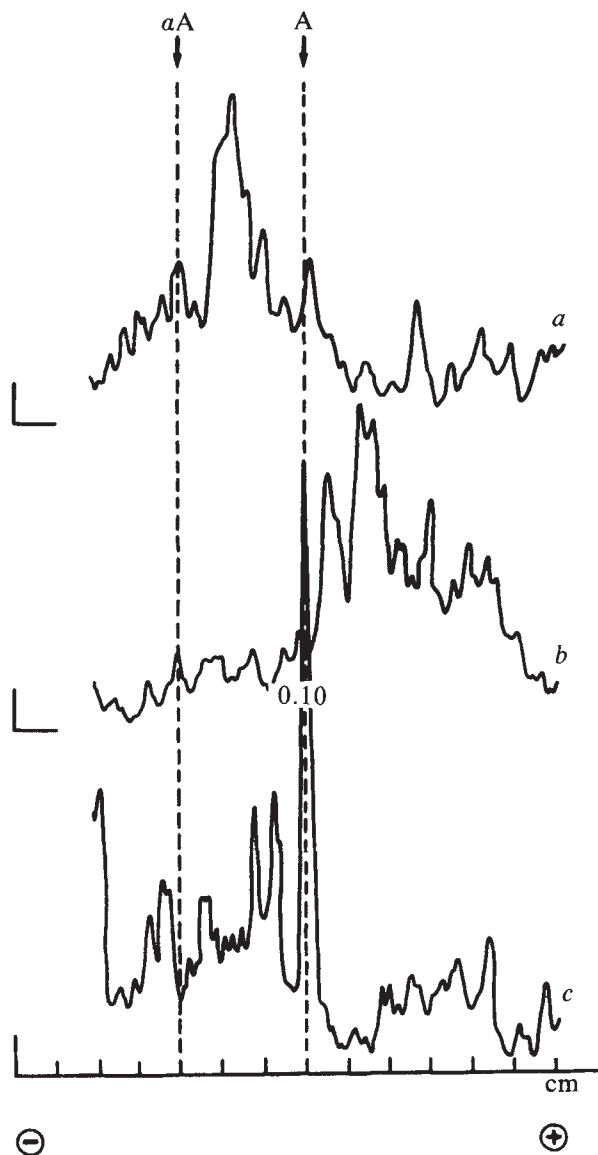


Fig. 2 Densitometer scans of membrane polypeptides stained after electrophoresis on SDS-polyacrylamide slab gels. Bovine adrenal chromaffin granules were prepared in sucrose buffered with 0.02 M 2-(*N*-morpholino) ethane-sulphonic acid (MES), with or without 0.15 M KCl (ref. 6). 5-HT granules and mitochondria were isolated from rabbit blood platelets using a continuous Urografin (Schering, West Germany) gradient as previously described²⁰. Membranes from chromaffin and 5-HT granules were prepared by lysing and washing the vesicles in buffer without sucrose. They were then solubilised by boiling them in 1% SDS for 2 min, subsequently subjected to gel electrophoresis²², and stained with Coomassie Brilliant Blue. *a*, Chromaffin granule membranes from vesicles isolated in 0.15 M KCl; *b*, 5-HT granule membranes; *c*, platelet mitochondria proteins. The arrows indicate the positions of α -actinin (α -A) and actin (A) standards applied to the same gel.

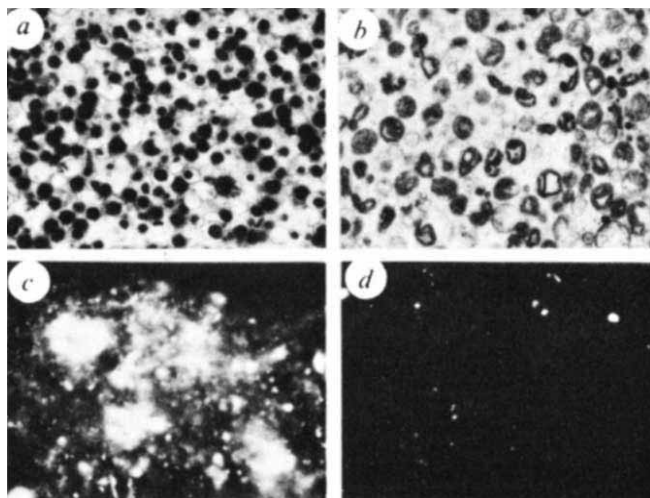


Fig. 3 Electron micrographs (*a, b*) and immunofluorescence staining (*c, d*) of platelet 5-HT granules (*a, c*) and platelet mitochondria (*b, d*). Aliquots of the same gradients were used. For electron microscopy, the material was fixed in 3% glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated and embedded in Epon 812. Ultrathin sections stained with uranyl acetate and lead citrate were examined with a Philips 300 electron microscope. Indirect immunofluorescence was performed on 4- μ m sections obtained by cutting the frozen material on a cryostat. The incubation procedure with the first (monospecific anti- α -actinin) and the second (FITC-GAR) antibody is described in Fig. 1. Photographs were taken on a Zeiss UV photomicroscope with epi-illumination using Anscochrome colour slide film 500 daylight (Gaf Corporation, New York) or HP4 Ilford film. Magnification: *a, b*, $\times 10,000$; *c, d*, $\times 400$.

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B. M. JOCKUSCH
M. M. BURGER

Biocenter,
University of Basel,
Basel, Switzerland

M. DA PRADA
J. G. RICHARDS

Research Division,
F. Hoffmann-La Roche & Co. Ltd,
Basel, Switzerland

C. CHAPONNIER
G. GABBIANI

Department of Pathology,
University of Geneva,
Geneva, Switzerland

Received 3 June; accepted 28 October 1977.

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Direct association of Balbiani ring 75S RNA with membranes of the endoplasmic reticulum

Puffs in polytene chromosomes probably represent active genes (see, for example, ref. 1). Cytogenetic investigations with large tissue-specific puffs, the Balbiani rings (BR) in *Chironomus* salivary gland cells, revealed a correlation

between the BR and salivary export proteins^{2,3}. Biochemical analysis of the BR showed that in *Chironomus tentans* RNA of a defined size is synthesised and transported to the cytoplasm⁴. This RNA, which sediments as 75S RNA, has properties of a messenger RNA, that it, it contains poly A⁵ and is located in polysomes⁶. If the BR-RNA directs synthesis of export proteins it would be expected to associate with the membranes of the endoplasmic reticulum (ER). Here we show that such an association exists and that it is independent of ribosomes and polysomal structures.

Labelled salivary gland RNA was obtained by micro-injection of tritiated RNA precursors 24 h before sacrifice of the animal. The labelling procedure has the character of a pulse which has declined 1–2 d after injection⁷. At 18 h the nucleus contains 2–4% of total labelled RNA and at 6 d it contained 1%. Thus from 18 h and onwards the risk of contamination of the cytoplasm with labelled nuclear RNA is very low or insignificant. Also, since the half-life of 75S RNA is about 20 h (Edström, J.-E., Lindgren, S., Lönn, U. and Rydlander, L., in preparation) analysis of these animals sacrificed 24 h after injection should give representative samples of the cytoplasm.

The distribution of total labelled RNA in agarose gel electrophoresis is shown in Fig. 1a: over 95% of the RNA visualised in this way is cytoplasmic RNA. The major constituents are the two ribosomal RNA species (28S and 18S RNA) and 4S RNA. There is also a prominent mono-disperse 75S RNA peak of Balbiani ring origin.

The salivary glands were homogenised in low salt buffer containing Mg²⁺ and the microsomal membranes pelleted by differential centrifugation⁸. It is difficult to keep 75S RNA intact during aqueous fractionation of the salivary gland cells. But, if sucrose is omitted and the centrifugation times scaled down, as in the procedure described here, there is very little, or no degradation of the RNA. This is evident if one compares the labelled RNA from glands homogenised directly in detergent (Fig. 1a) with those fractionated in low salt buffer before detergent treatment (Fig. 1b). In

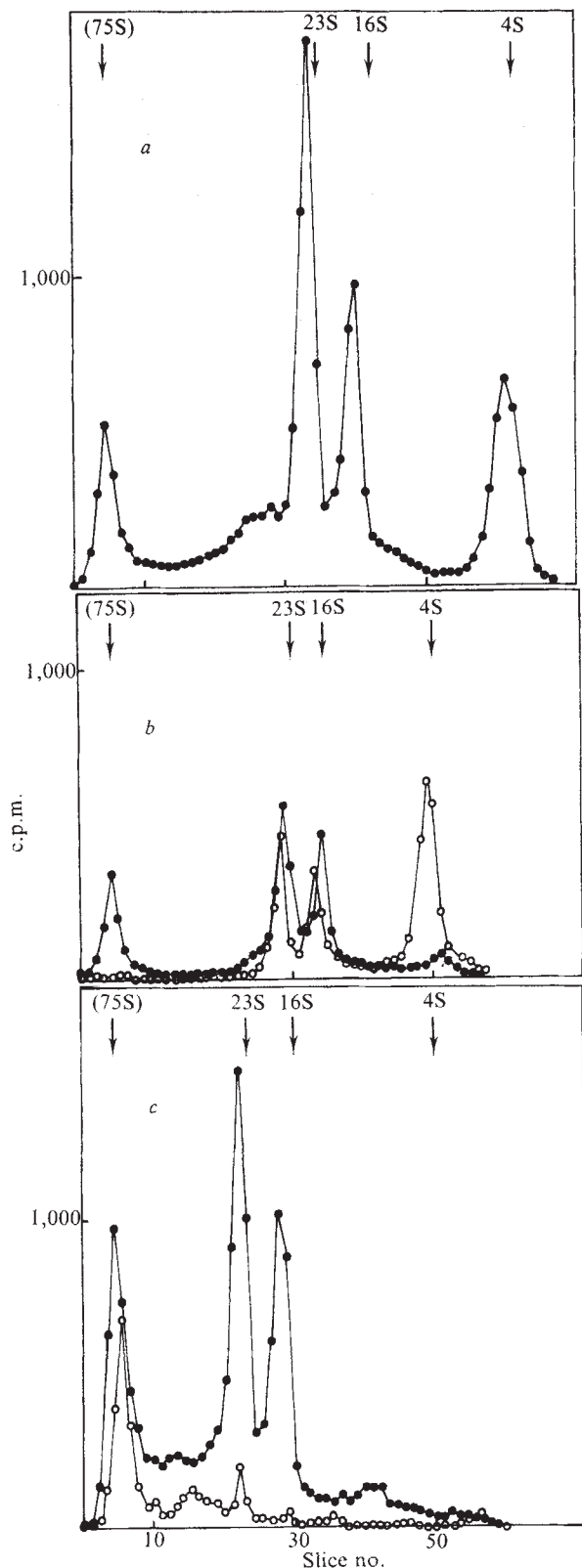


Fig. 1 a, Larvae of *Chironomus tentans* were injected with 25 μ Ci tritiated uridine (50 Ci mmol⁻¹; Amersham Radiochemical Centre) 24 h before killing. The excised salivary glands from two animals were homogenised in 0.5 ml 1% sodium dodecyl sulphate (SDS) in 0.02M Tris-HCl pH 7.4 for 5 min at 25°C. The RNA was ethanol-precipitated and separated in 1.5% agarose slab gels¹². *E. coli* RNA was used as carrier (23S, 16S and 4S RNA). b, To determine the amount of membrane-bound 75S RNA a procedure that separates membranes from cytosol was used⁸. The excised salivary glands were allowed to swell for a couple of minutes in 0.5 ml cold reticulocyte standard buffer (1.5 mM MgCl₂, 10 mM KCl, 10 mM Tris-HCl pH 7.4) and homogenised in a Potter-Elvehjem glass homogeniser. To remove polytene chromosomes and so on the homogenate was centrifuged for 2 min at 750g_{max} and to pellet the membranes the supernate thus obtained was centrifuged for another 5 min at 27,000g_{max}. To release RNA the 27,000g_{max} supernate was made 1% with respect to SDS, whereas the pellet was extracted by addition of 0.5 ml 1% SDS in 0.02 M Tris-HCl pH 7.4. The samples were ethanol-precipitated after 5 min at 25°C. Electrophoresis was performed in 1.5% agarose slab gels¹². ●, 27,000g_{max} pellet; ○, 27,000g_{max} supernate. Earlier control experiments showed that 87% of labelled phospholipids appeared in the 27,000g_{max} pellet⁸. Also, over 80% of total labelled heavy ribosomal subunits appeared in the pellet at steady-state distribution (6 d after precursor injection)⁸, whereas after 24 h only about 50% appeared in the 27,000g_{max} pellet. c, To release ribosomes from the microsomal membranes a homogenate prepared as in b was divided into two equal aliquots, one of which served as a control. Cold buffer was added to the control aliquot to restore the original volume and immediately centrifuged to pellet the microsomal membranes. To the other aliquot an equal volume of high salt buffer was added (2 M KCl, 50 mM Tris-HCl pH 7.4, 2 mM puromycin, 10 mM EDTA⁹). After incubation for 30 min at 4°C the membranes were pelleted. The pellets were extracted with 1% SDS and the RNA separated in 1.5% agarose slab gels. ○, Stripped membranes; ●, control membranes.

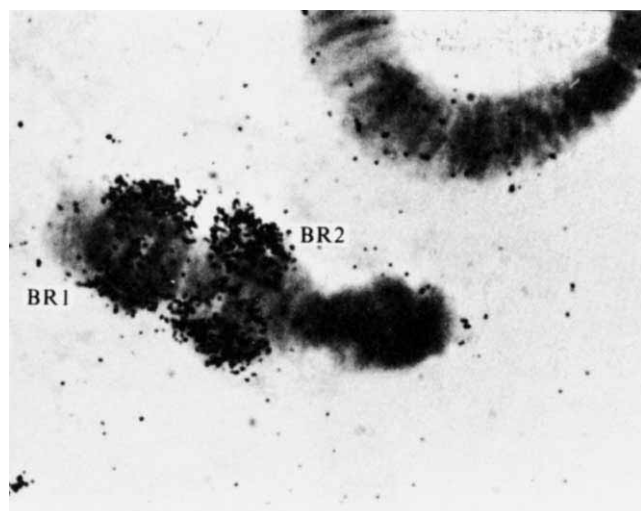


Fig. 2 Autoradiograph of chromosome IV of *Chironomus tentans* salivary gland cells after *in situ* hybridisation with RNA from stripped membranes. The RNA sample, obtained from membranes stripped of ribosomes as in Fig. 1c and passed through a Sephadex G-25 column, was dissolved in 15 μ l $2\times$ SSC (SSC is 0.15 M sodium chloride and 0.015 M sodium citrate) and added to salivary gland squashes. The squashes were previously denatured in 90% formamide in $0.1\times$ SSC at 62–64 °C for 2 h. The hybridisation reaction was carried out at 62–64 °C for 4 h. The subsequent treatments (RNase-treatment, washing in $2\times$ SSC and dehydration) was done according to ref. 13. Autoradiography was performed with Kodak AR 10 stripping film; exposure time was 12 weeks. The length of chromosome IV is about 80 μ m.

control experiments, heating the RNA sample in 8 M urea caused no significant degradation of 75S RNA⁸.

The distribution of labelled RNA between the membrane pellet and its corresponding supernatant is shown in Fig. 1b. The membrane pellet contained 75S RNA and two ribosomal RNA species (28S and 18S RNA). The supernate contains in addition to these RNA species also 4S RNA. The major part (more than 90%) of 75S RNA appeared in the membrane-fraction.

Further experiments were performed to establish whether it is possible to release ribosomes from the microsomal membranes still keeping the 75S RNA associated to the membranes. To strip the membranes free of ribosomes the membranes were treated *in vitro* with high ionic strength KCl buffers with EDTA and puromycin for 30 min at +4 °C (ref. 9). Gel electrophoresis showed that there was a nearly complete removal of 18S RNA from the membranes. The 28S RNA was removed to 80–90% but over 50% of the 75S RNA remained attached to the membranes (Fig. 1c). Control experiments showed that less than 10% of the 75S RNA appeared in the membrane pellet when it was treated with detergent (0.5% DOC and 0.5% Tween 80) and recentrifuged.

In situ hybridisation with labelled RNA from membranes treated with KCl-EDTA-puromycin showed grains over BR one and two (Fig. 2). No other loci, including the nucleolar region, hybridised significantly. In controls with total RNA, also the nucleolar region hybridised significantly. Thus RNA with sequences complementary to the DNA of BR one and two is located in the membrane pellet after the complete release of 18S RNA and the release of the major part of 28S RNA. This indicates that there is in all probability a 75S RNA-membrane interaction which is independent of ribosomes and polysomal structures.

Similar findings have been obtained in fibroblast¹⁰, liver⁹ and HeLa cells¹¹ where the attachment of mRNA to microsomal membranes was shown to exist via the 3'-end containing the poly A tail. 75S RNA also contains poly A (ref. 5) but it is not known whether it is responsible for the attachment.

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ULF LÖNN

Department of Histology,
Karolinska Institutet,
S-104 01 Stockholm, Sweden

Received 18 August; accepted 17 October 1977.

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Subgenomic, cellular Rous sarcoma virus RNAs contain oligonucleotides from the 3' half and the 5' terminus of virion RNA

THE genome of Rous sarcoma virus (RSV) is a 30–40S RNA of 10,000 nucleotides coding for four known genes¹ which map in the order 5'-*gag*, *pol*, *env*, *src*, poly(A)-3' (refs 2, 3). This 30–40S RNA serves as mRNA for a *gag* precursor protein and perhaps a *gag-pol* precursor-polypeptide when translated *in vitro*^{4,5}. The *env* gene, however, may be translated from a smaller intracellular messenger. Poly(A)-containing 20–24S cellular RNA from infected cells can, upon microinjection, complement an *env*-defective virus⁶ and may also direct *in vitro* synthesis of a protein that is serologically related to the *env* gene product⁷. Moreover, hybridisation with DNA complementary (cDNA) to *env* and *src* sequences of virion RNA detects discrete classes of subgenomic poly(A)-containing RNAs present in infected cells as steady state species, while *gag* and *pol*-specific cDNAs hybridise only to full sized RNA^{8,9}.

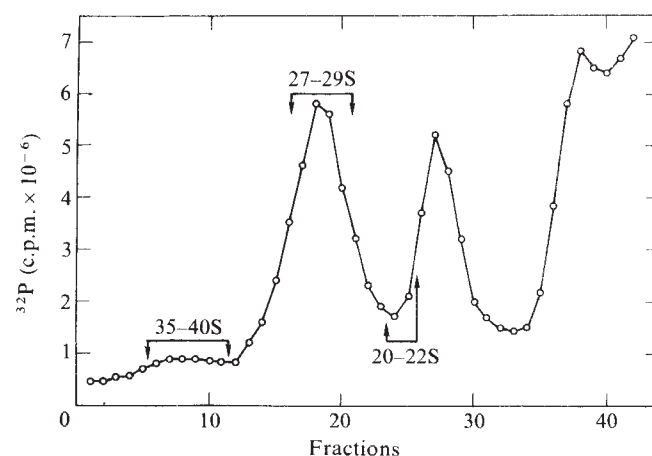


Fig. 1 Sucrose gradient sedimentation of cellular RNA from RSV-infected cells. PR-RSV-B-infected cells^{2,3,12,13} labelled with 75 mCi (³²PO₄)³⁻ for 3 h were trypsinised, suspended in 0.1 M NaCl, 0.01 M Tris (7.4), 1 mM EDTA, extracted twice with phenol, chloroform, Na₂SO₄, mercaptoethanol, at about 60:38:1:1, and ethanol-precipitated. Nucleic acids were heated to 100 °C in 0.01 M NaCl, 0.01 M Tris (7.4), 1 mM EDTA with 0.1% Na₂SO₄ and layered onto a gradient of 10–25% sucrose containing the same buffer. Centrifugation was for 6.5 h at 40,000 r.p.m. and 20 °C in an SW41 rotor. Radioactivity was Cerenkov counted and the RNA of the three pools indicated was analysed as described in Fig. 2.

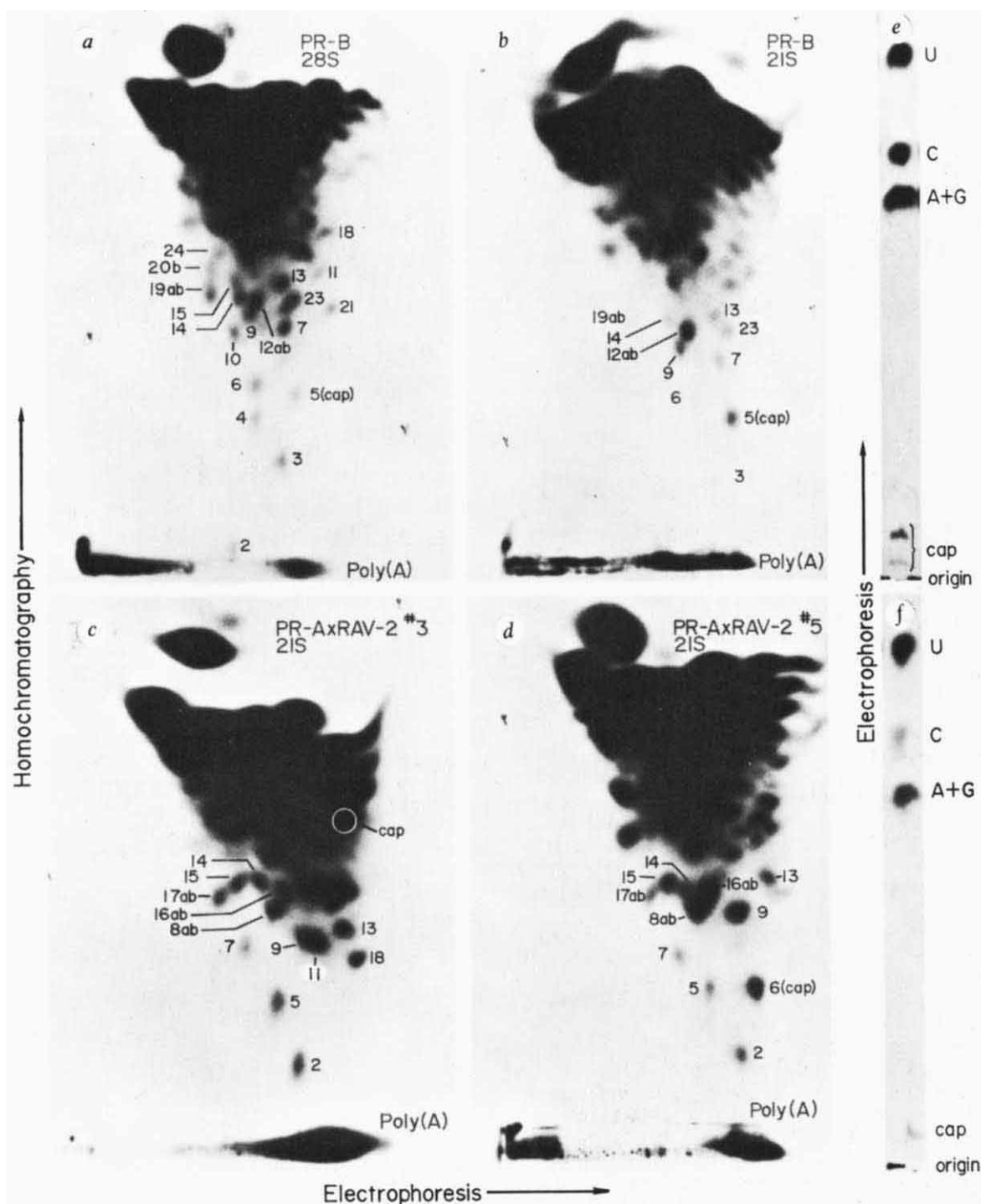


Fig. 2 Two-dimensional fingerprint analysis of poly(A)-tagged, virus-specific, intracellular RNA and analyses of their 5' terminal, capped-oligonucleotides. Three different size classes of poly(A)-tagged RNA species, isolated from about 5×10^7 RSV-cells, as for Fig. 1, were selected by chromatography on oligo(dT)-cellulose as described^{2,3,13,12,13}. The RNA of each pool, about $6 \cdot 10 \times 10^6$ c.p.m., was mixed with 0.7 μ g viral poly(dC)-cDNA and heated to 100 °C in 0.01 M NaCl, 0.01 M Tris HCl, pH 7.4, 1 mM EDTA and lyophilised. The mixture was then incubated in 25–50 μ l 70% formamide, 0.3 M NaCl, 0.03 M Na citrate, 1.5 mM Na phosphate, pH 7.0, at 40 °C for 6–10 h. Subsequently the solution was diluted with 1 ml 0.15 M NaCl, 0.015 M Na citrate and heated at 60 °C for 10 min. RNA-DNA hybrids were selected by chromatography on a 1 ml-column of oligo(dG)-cellulose (Collaborative Research, Waltham, Mass.) by a modification of procedures described previously^{20,21}. The conditions were as described for chromatography on oligo(dT)-cellulose, except that the column was washed with 15 μ g poly(rA) in 1 ml application buffer (0.5 M LiCl, 0.01 M Tris HCl, pH 7.4, 0.05% Na dod SO₄) and rinsed with 0.5 ml of the same buffer lacking poly(rA) prior to chromatography of the hybrids. The eluted hybrids were made 0.5 M LiCl and rechromatographed. The hybrids were melted by heating to 100 °C at low ionic strength, ethanol-precipitated and the hybridisation-selection process repeated except that only 0.1–0.2 μ g poly(dC)-cDNA was added and that the hybrids were selected only once on the oligo(dG)-cellulose column. Recovery of poly(A)-tagged RNA as hybrids was between 0.05 and 4% for distinct pools (see text). Eluted hybrids were melted as above and ethanol-precipitated for T₁ digestion and two-dimensional fingerprinting^{12,13,15}. PR-B cDNA was used in all experiments. It was synthesised as described²² and prepared by exclusion chromatography on a Biogel P100 (12 $\times$ 0.7 cm, BioRad, Richmond, CA) column in 0.01 M Tris, pH 7.4, 1 mM EDTA and 0.05% Na dod SO₄. About 6 μ g cDNA was polycytidylated with 2 units of deoxynucleotidyl transferase (PL-Biochemicals) in 60 μ l 0.1 M KH₂PO₄, 0.05 M cacodylic acid, pH 7.0, 1 mM mercaptoethanol, 2 mM CoCl₂, 3 mM dCTP for 12 h at 40 °C. Poly(dC)-cDNA was purified by chromatography on Biogel P100 as above. Excluded cDNA was then selected on oligo(dG)-cellulose as described above omitting poly(rA). Recovery of poly(dC)-cDNA was 65%. Panels A–D are autoradiographs of T₁-resistant oligonucleotides of virus-specific RNAs resolved by two-dimensional electrophoresis-homochromatography^{2,3,12,13}, termed fingerprinting. (a) Finger-

Neither the structure nor the mechanism of generation of these subgenomic RNA species has been elucidated. They may be produced by partial transcription of the DNA provirus or by functionally specific cleavage of genome-sized RNA. They may also include species generated by structurally specific degradation of viral RNAs. The structure of such RNAs would be that of a contiguous segment of virion RNA with at least one terminus derived from an internal location on the virion RNA. Alternatively, subgenomic viral RNAs could be generated by internal deletions of virion RNA, a mechanism analogous to that which has been discovered to govern the mRNA synthesis of adenovirus, a DNA-containing virus. Some adenovirus mRNAs were found to have a 100–200 nucleotide sequence covalently attached to the 5' end of the coding sequence, but transcribed from a remote segment of the DNA template^{10,11}. This observation suggests that covalent linkage of transcripts from non-adjacent DNA templates, termed splicing, may be characteristic of eukaryotic cellular and viral mRNAs.

We have isolated and characterised the poly(A)-tagged RSV-specific RNAs synthesised during a 3-h ³²P labelling period in infected cells, specifically addressing the questions of sequence arrangement and derivation of their terminal sequences. These analyses were done using the large, unique RNase T₁-resistant oligonucleotides of virion RNA as diagnostic of distinct viral RNA segments^{12,13} for two reasons: (i) Since their order relative to the poly(A)-terminus of each viral RNA studied here is already known^{2,3,12}, their detection would directly identify those virion RNA segments from which a subgenomic RNA species was derived. (ii) Moreover identification of the 5'-terminal 7mGpppGmC(cap)-oligonucleotide^{14,15} of a subgenomic viral RNA species would unambiguously define its 5'-terminus. This cannot be done directly by hybridisation with cDNAs complementary to the termini of virion RNA, because such cDNA detects presumably redundant sequences on both ends of virion RNA in addition to internal sequences^{16,17,18} and because positive hybridisation does not indicate where on the RNA a given sequence is located.

Viral-specific poly(A)-containing RNA was isolated from Prague RSV-B (PR-B)-infected cells by the following procedure: RSV-transformed fibroblasts were incubated with media containing [³²PO₄]³⁻ for 3 h. Nucleic acids, phenol-extracted from these cells, were fractionated by sucrose gradient sedimentation (Fig. 1). Three size classes marked in Fig. 1 were pooled and poly(A)-containing RNA species were selected by chromatography on oligo(dT)-cellulose. The recoveries were 16% for the 35–40S pool, 4% for the 27–29S pool and 20% for the 20–22S pool. Virus-specific RNA was isolated by twice repeated hybridisation to poly(dC)-tagged PR-B cDNA with selection on oligo(dG)-cellulose (see Fig. 2). The recovery of [³²P]RNA from each original pool was approximately 4% (35–40S), 0.1% (27–29S) and 0.3% (20–22S). The percent virus-specific [³²P]RNA in the 27–29S and 20–22S species prepared by hybridisation to viral cDNA was 60–80% as determined by resistance to digestion with

with RNase T₁ and analysed by two-dimensional fingerprinting^{2,3,12,13}. Each large T₁-resistant oligonucleotide was identified by its relative chromatographic location and by analysis of its RNase A-resistant fragments (not shown) and numbered as its defined counterpart in virion RNA^{2,3,13}. In every case the 35–40S pool was identical to virion RNA (not shown). The T₁-oligonucleotides present, at approximately equimolar ratios, in the 27–29S intracellular PR-B RNA (Fig. 2a) are those underlined and oligonucleotides present in the 20–22S RNA (Fig. 2b) are those shown in bold face type on the complete oligonucleotide map of PR-B RNA: nucleotide (ref. 24) of the 20–22S PR-B RNA gave the following near equimolar amounts expressed as c.p.m. per phosphate: 5(cap): 3.5, 13: 2.5, 23: 3.4, 19ab: 1.3: 14: 3.6, 7: 2.9, 9: 3.6, 12ab: 4.8. All of the oligonucleotides of the **5(cap)**, 17, 16, 8, 22, 20a, 11, 4, 20b, 15, 2, 10, 21, 18, **6, 3, 13, 23, 19ab, 14, 7, 9, 12ab, C, Poly(A)** (ref. 2).

Quantitation of the radioactivity in each major oligonucleotide (ref. 24) of the 20–22S PRB RNA gave the following near equimolar amounts expressed as c.p.m. per phosphate: 5(cap): 3.5, 13: 2.5, 23: 3.4, 19 ab: 1.3, 14: 3.6, 7: 2.9, 9: 3.6, 12 ab: 4.8. All of the oligonucleotides of the two subgenomic RNA species are derived from the 3' half of the viral oligonucleotide map except for oligonucleotide no. 5. We must, however, emphasise that the order of the oligonucleotides of the subgenomic RNA species cannot be deduced from our data, except that of the 5'-terminal cap-oligonucleotide, no. 5. This oligonucleotide was recovered from 20–22S RNA (Fig. 2b) and shown to contain the cap-structure (Fig. 2e) and to have the same RNase A-resistant fragments as the cap-oligonucleotide of virion RNA^{14,15} (data not shown).

The presence of the 5'-terminal cap-oligonucleotide of virion RNA in subgenomic viral RNA species could reflect a splicing mechanism for the synthesis of subgenomic viral RNAs. Alternatively the viral genome could contain internal copies of this oligonucleotide. To distinguish between these alternatives we have analysed the 20–22S viral-specific RNA from cells infected by two recombinant RSVs. These recombinants have essentially indistinguishable oligonucleotide maps except for their 5'-terminal cap-oligonucleotides¹⁹. PR-A × RAV-2 no. 3 contains a RAV-2-derived cap-oligonucleotide, which chromatographs with hexanucleotides in a two-dimensional fingerprint¹⁹. The T₁-oligonucleotides detected in the 20–22S intracellular RNA of this recombinant (Fig. 2c) are those shown in bold face type in the following oligonucleotide map of the viral RNA (ref. 2): **RAV-2-cap**, 15, 16c, 10, 12, 19, 21, 4, 20, 3, 33, 18, 11, 23, **5, 7, 2, 8b, 13, 17ab, 14, 16ab, 9, 8a, C, poly(A)**. The c.p.m. per phosphate of the major oligonucleotides were: 15: 9.1, 11: 4.7, 5: 7.0, 7: 6.0, 2: 5.2, 8ab: 4.5, 13: 8.9, 17ab: 4.4, 14: 8.9, 16ab: 6.7, 9: 7.8. The presence of the cap-structure in the oligonucleotide-spot marked 'cap' in Fig. 2c is shown in Fig. 2f. PR-A × RAV-2 no. 5 contains a PR-A-derived cap-oligonucleotide, that is, no. 6, which is identical to that of PR-B (ref. 15). (Note, the map location of no. 6 has been revised. It was originally confused with no. 9, [ref. 2].) Figure 2d shows that the 20–22S intracellular RNA of recombinant no. 5 contains the cap-oligonucleotide no. 6 and all those oligonucleotides of virion RNA which are shown in bold face type in the following map (ref. 2): **6-cap**, 15, 16c, 10, 12, 19, 21, 4, 20, 3, 11, 18, **7, 5, 2, 8b, 13, 17ab, 14, 16ab, 8a, 9, C, poly(A)**. The c.p.m. per phosphate of the major oligonucleotides were: 6(cap): 8.2, 15: 12.5, 7: 5.4, 5: 4.5, 2: 4.0, 8ab: 11.7, 13: 7.7, 17ab: 3.4, 14: 6.2, 16ab: 15.0, 9: 12.8. The 20–22S species of both recombinants also contained RAV-2 oligonucleotide no. 15 which maps near the 5' end of the virion RNA but does not contain the cap-structure. Thus, because the 5' cap-oligonucleotides of the 20–22S RNA species of PR-B, PR-A × RAV-2 no. 3 and no. 5 were identical to cap-oligonucleotides of their

print of 27–29S RNA of PR-B infected cells. (b) 20–22S RNA of PR-B infected cells. (c) 20–22S RNA of PR-A × RAV-2 no. 3 infected cells. (d) 20–22S RNA of PR-A × RAV-2 no. 5 infected cells. All numbered oligonucleotides were identified as identical to virion RNA counterparts (see text). Panels E and F are autoradiographs of RNase A, T₁- and T₂-resistant fragments of oligonucleotide spots expected to contain 7mGpppGmC(cap)-structures, after electrophoresis on DEAE paper at pH 3.5 (ref. 15). e, The nuclease-resistant fragments of oligonucleotide no. 5 of 20–22S PR-B RNA, recovered from the fingerprint shown in (b). f, The nuclease-resistant fragments of the oligonucleotide spot, marked 'cap' of 20–22S PR-A × RAV-2 recombinant no. 3 RNA recovered from the fingerprint shown in c.

RNases T₁, T₂ and A after hybridisation with an excess (10–50-fold) of viral cDNA. These RNA pools were digested

respective virion RNAs, and the 20–22S of the two recombinants also contained a unique internal oligonucleotide (no. 15) that maps close to the 5' end of each recombinant RNA, the 5' segment of each 20–22S RNA species must have been transcribed from a proviral DNA segment corresponding to the 5' end of virion RNA.

A minimum estimate for the length of the 5' segment is 16 nucleotides (oligonucleotide no. 15, ref. 2) plus 22 nucleotides (large cap-oligonucleotide) or 37 nucleotides. This segment, however, is likely to be longer because recombination occurs between oligonucleotide no. 15 and the 5' end (as in recombinant no. 5) and because oligonucleotide no. 15 also occurs at the 5' end of RSV-B77¹² though it is not found in the first 110 nucleotides as sequenced by Shine *et al.*¹³.

It may be argued that the viral 5' oligonucleotides in the 20–22S cellular species were derived from minor contaminants of intact or degraded virion RNA selected by our cDNA rather than from a subgenomic 20–22S RNA. Although, our cDNA contained typically (ref. 23) three- to five-fold more sequences complementary to the 5' oligonucleotides than to other oligonucleotides (determined by hybridisation to an excess of ³²P-viral RNA and fingerprinting of RNase T₁-resistant hybrids), this possibility has been ruled out for the following reasons (data not shown). The fingerprints of 35–40S viral RNA isolated from infected cells had an equimolar representation of all oligonucleotides indicating no bias in the selection process. In addition, if our PR-B poly(dC)cDNA was hybridised to a 10-fold excess of 30–40S ³²P-viral RNA and the hybrids were selected and fingerprinted as in Fig. 2 all PR-B oligonucleotides were approximately equimolar. This is because asymmetrical hybrids consisting of small, approximately 5S cDNA and large viral RNAs sedimenting between 10 and 35S, with a peak at 18S, were selected for fingerprint analysis by our method. Since the cellular viral RNA species analysed in this paper were also large after isolation (average approximately 18S), oligonucleotides adjacent to the 5' terminus should have also been detected if the 5'-terminal oligonucleotides were derived from contaminating intact or degraded viral RNA species.

We conclude that RSV-infected cells contain specific viral RNA species which consist of a capped segment from the 5' end of virion RNA attached to a polyadenylated longer segment from the 3' end. This suggests that subgenomic RSV RNAs are synthesised by a splicing mechanism analogous to that observed in adenovirus or perhaps by transcription from specifically deleted proviral DNAs. The data argue against subgenomic RNAs being generated by cleavage of 35–40S RNA or by internal transcription of full-length provirus. Further work is necessary to establish whether these subgenomic viral RNAs function as mRNAs.

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PAMELA MELLON
PETER H. DUESBERG

Department of Molecular Biology and Virus Laboratory,
University of California,
Berkeley, California 94720

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Errata

In the letter 'Structures of benzo(a)pyrene-nucleic acid formed in human and bovine bronchial explants' by A. M. Jeffrey *et al.*, *Nature* 269, p. 348, lines 25–26 in Fig. 1 legend should read . . . poly (G) were detected by ultraviolet absorbance at 280 nm and *in vivo* samples by their radioactivity. Line 19 in Fig. 2 legend should read . . . *in vivo* product was obtained (upper panel, *b*) which, when reanalysed . . . Line 2 in Fig. 3 legend should read . . . corresponding to the major *in vivo* DNA adduct from human and . . . Line 9 in Fig. 3 should read . . . (shaded area at 350–360 nm, 'L_b, short axis; 310–355, 'L_a, long. . .

In the letter by R. G. Strom and D. E. Harris, *Nature* 269 581–582 (1977), the title should read 'HD26676: Radio emission from a normal star'. On p. 581 paragraph 3 line 12, for 'Westerbork' read 'Westerbork'. In paragraph 4 line 5 for 'frequency α ' read 'frequency'.

In the letter 'Long-range attraction between red cells and a hydrocarbon surface' by D. Gingell and I. Todd, *Nature* 268, p. 767, two lines have been omitted. Line 12 in paragraph 2 should read . . . the oil/water interface was measured by the hanging drop. . . Line 28 in paragraph 4 should read . . . of attraction from 140 nm to 450 nm. The only kind. . .

Corrigenda

In the letter 'Structures of benzo(a)pyrene-nucleic acid formed in human and bovine bronchial explants' by A. M. Jeffrey *et al.*, *Nature* 269, p. 348, in Fig. 2 legend line 18 for 50–60% methanol read 50–65% methanol.

In the letter 'Surge activity on the Barnes Ice Cap' by G. Holdsworth, *Nature* 269, p. 588, the journal in ref. 14 should be *J. appl. Met.*

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christmas Books supplement

Images of animals

Colin Blakemore

Animals and Men: Their Relationships as Reflected in Western Art from Prehistory to the Present Day. By Kenneth Clark. Pp. 240. (Thames and Hudson: London, 1977.) £10.50.

EVERYTHING about this book is full of promise. Its author, Lord Clark, is now a cult object more widely revered than Horus and Hathor, the sacred falcon and cow of ancient Egypt who decorate several of the book's ample pages. The title is provocative and challenging. The book is (literally) weighty and the many, many illustrations are generally of high quality. Most of all, the timing is impeccable. It is surely no accident that this elegant volume appears a few weeks before Christmas in a year of intense public interest in the status of animals and the need for their protection. Not only is there the growing public sentiment for the conservationist movement, but there is also heightened anti-vivisectionist activity in response to the centennial of the British Cruelty to Animals Act. There can be no more convincing sign of the times than the growing power of the 'Ecology' movement, even in France—that bastion of effortless pragmatism, where there has always been more appetite for, than love of, animals.

Despite the richness of its potential and the certainty of its commercial success, the book in actual fact does not fulfil its promise. Lord Clark's text is merely a brief essay, which at times is no more than a list of the plates that follow it. The captions to the illustrations, which enlarge a little on the text, were not even written by Lord Clark but by three assistants. Most disappointing of all is the fact that the book is *not* primarily about the relationship between animals and man: it is largely a catalogue of western works of art that happen to contain images of animals.

Text and plates are arranged around five subjects: "Sacred and Symbolic Animals"; "Animals Observed"; "Beauty and Energy of Animals"; "Animals Beloved"; and "Animals Destroyed". A scholar of Lord Clark's academic stature, vast experience, and skill in communication is expected to bring insight and even humour to these topics. And he does. The balance between admiration, curiosity and love in our relationship with animals is nicely handled. And there are fascinating vignettes—like the fact that Leonardo was

a vegetarian; that Cezanne, Monet and Manet never painted an animal; and that for 700 years all animals in art were symbols for the evangelists.

But the closest that Lord Clark comes to deeper issues is on the very first page, where he asks why the mythical harmony



of animals and man in the Golden Age never actually came to pass. "The answer lies in that faculty which was once considered man's highest attainment, a gradual realization that the sounds he uttered could be so articulated as to describe experience. He discovered words, he could communicate with other men."

This idea (that language bluntly separates man and beast) recurs from time to time. To the Egyptians, the haughty speechlessness of animals made them worthy of worship. And we are said to play with animals because in doing so "we forget the difference that separates us—the faculty of speech. Children would rather play with a teddy-bear than with a small model human being, and they put long imaginary speeches into the bear's mouth. Thus, the barrier between animals and man is broken down". Quite apart from the fact that Lord Clark does not seem to have heard of dolls, I do not trust the hypothesis that people are truly distinguished from animals by their speech, any more than the suggestion that the relationships between individual human beings rest on language alone.

What of the remarkable communion that can exist between a rider and his horse, between a farmer and his herd,

between people and their pets? And, equally, what is it that makes us wince at the mindless existence of a battery hen or bristle with anger when we see magnificent animals languishing in tiny cages? I believe that our extraordinary empathy with animals springs from the fact that we are animals too. Animals with insight enough to recognise our biological oneness with other beasts.

And yet our animal nature, as well as making us admire and understand the special skills of other species, also drives us to kill and eat a number of them, because that is what we, as animals, are clearly designed to do. Lord Clark certainly touches on this paradox in our attitude to animals. "We love animals, we watch them with delight, we study their habits with ever-increasing curiosity; and we destroy them." But he does not face squarely the question of man as an animal of such cultural sophistication that he can use a representation of another species as an exercise in the exploration of his own deepest emotions.

Even as an animal picture-book, it has some surprising omissions. Where are Henri Rousseau's strange jungle beasts with their haunting eyes; the mysterious animal automata of Maurits Escher; and the folksy farmyard beasts of Marc Chagall? But more important, where are the works of art that explore the real question of man *as* animal? Goya's terrifying masterpiece of *Saturn Devouring his Children*, the Tiepolo *Martyrdom of St John of Bergamo* (what a chance to compare it with the Delacroix or Rubens' *Lion Hunts*); the mythical man-beasts of Michael Ayrton; and the lumps of animal flesh that pass for portraits by Francis Bacon?

Lord Clark explains in a Foreword that this enterprise grew out of a commission for a book on animals in art from the World Wildlife Fund (which will receive a contribution from its sales). "This by itself did not seem to me to constitute a subject," writes Lord Clark, "but on reflection it occurred to me that no-one had ever given much thought to the relationship of animals and men, and that this might be a subject worth exploring." Of that there is no doubt. Let us hope that it will continue to be explored as part of the effort to establish the true nature of man. □

Colin Blakemore is Royal Society Locke Research Fellow at the Physiological Laboratory, University of Cambridge, UK.

Francis Barlow's frontispiece to his edition of Aesop's Fables, 1665

Taken from *Animals and Men*

Birds of prey, owls and seabirds

WHY birds of prey should be so popular is rather difficult to fathom, for they are not exceptionally popular with bird-watchers and there are many bird-lovers and perhaps shooters who would think of them as "cruel killers". Perhaps supporters of birds of prey are just prolific writers, for each year they become the subject of several books. Last Christmas three books came to me for review; this year, they have retained their popularity with another three. Sometimes, one finds the same sort of text with the same facts and theories possibly presented in a slightly different way. Whether the texts are original or 're-hashes' depends on how far into the basic papers the popular writer is prepared to read. Two of the three books on birds of prey in this selection are written by well established raptor enthusiasts: Michael Everett writes on the *Natural History of Owls* (Hamlyn: London and New York; £3.50) and Lea MacNally provides an individual account of *The Ways of an Eagle* (Collins: London; £5.95).

The *Natural History of Owls* is a 'popular', large format book, well written

and well researched, with many photographs chiefly in black-and-white: there may be some sense in this because plate after plate of brown and grey owls tend to become monotonous anyway. Also, most owls conform to much the same physical pattern of a non-specialist predator, which are most highly developed towards living in the dark. Everett provides particularly good reviews of the chief hunting methods used by owls, of which there are relatively few, and of the work undertaken by many authors on the feeding ecology of a number of species. He also summarises the known complexities of the predator-prey cycle; these tend to become ever more complex where the bird's range covers many habitats. Studying nocturnal birds is particularly difficult, even though "image intensifiers" have occasionally been brought into use for certain aspects of research. The final chapter "Owls and Man" returns to a theme which has been much worked on over the years—their effect on superstitious Man. The author traces Man's reactions to owls from the time when the owl was regarded as a welcome neighbour, to the time of the so-called agricultural revolution in the mid-eighteenth century and the rise in field sports, when all hook-beaked birds, regardless of their known food preferences, were destroyed as enemies. It is only recently that scientists of the Nature Conservancy Council, the Institute of Terrestrial Ecology and the Royal Society for the Protection of Birds have begun to show with incontrovertible data how misleading have been the prejudices of some of the shooting landlords and their gamekeepers. In short, this book is a well written and easily read summary of what is known of the owls of the world.

Lea MacNally's *The Ways of an Eagle* is different in that it is based on personal experience with several pairs of eagles gained over 22 years as he followed his profession, first as a deer-stalker and latterly as a Warden of the National Trust for Scotland in north-west Ross-shire. The book is divided into four sections which deal with various aspects of the life history of the four pairs of eagles on which he had concentrated. The sections deal with the survival of the golden eagle, nesting and fledging, their food, behaviour throughout the season, and finally, four appendices detailing breeding success, weights of eaglets, lists of prey and nest material brought to the nest. One advantage that this type of book has over the monograph is that the real personal experience shines through, so that you get the feeling that you are really with MacNally in the hills. You savour more clearly the thrill of seeing these huge eagles, for which Britain is one of the most important—if not the most important—stronghold in the world.

Even though MacNally concentrates on the golden eagle this book is not a mono-



Great horned owl

Taken from Natural History of Owls

graph: he has not collected or attempted to synthesise the results of the enormous amount of research which has been undertaken on the golden eagle. Notwithstanding this, Lea MacNally gives us a very readable account of the ways of the golden eagle.

The Birds of Prey in Britain and Europe (Hamlyn: London and New York; £1.50) by Miroslav Bouchner and Dan Barta is a concise guide in colour, with a refreshingly different text written by a Czechoslovakian and presumably based on different sources from that available to English writers. The illustrations perform their function and enable one to identify raptors when observed in ideal conditions. There are also two useful plates showing the typical underviews of most species, but these do not of course indicate the range of plumage variation possible with birds of prey. Unfortunately, this book has no bibliography; so it would be difficult for the serious raptor enthusiast to trace statements to their original source.

Seabirds are another group of birds which have their adherents, and to cope with a popular demand from visitors to Britain's coastline Bruce Campbell has written *Birds of the Coast and Sea: Britain and Northern Europe* (Oxford University: Oxford; £3.75), which has been illustrated by 64 paintings by Raymond Watson. Campbell's text accompanies the paintings closely but he has also added shorter notes to describe the appearance and habits of the less common species which could appear but which have not been illustrated. Each specific text begins with a description of the plumage of each species, information about their geographical range and breeding distribution, their calls, breeding behaviour, size of population, and finally anecdotal accounts of interesting aspects of a bird's behaviour. The whole is well written and should provide ornithological companionship.

The illustrations do not reach the same standard as the text. The style of painting is representational, as far as the birds are concerned; Mr Watson does, however, avoid painting a bird's habitat in the same detail and so the bird appears on a misty shore lit by subdued light. He does not

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always get the shape of the bird quite right and often draws the bill rather too long. I think I might have trouble in trying to identify some of the small waders from these pictures but one or two of the pictures of gulls in flight have light, life and beauty.

My final comment on this book is that it is sad to see the text printed in such small type. Presumably it was used in a laudable attempt to keep the price down—which is reasonable as present prices go.

Another book which is not easy to read is the large format book with several photographs on each page. In *Wonderful World of Birds and their Behaviour*, by Donald Broom (Hamlyn: London and New York; £2.95), I get the impression that the designer was thinking more about designing the page to sell the book by its photographs than about the difficulty the reader might have in finding his way to the text through the jumble of pictures and legends. This lay-out I found particularly distracting, even though Donald Broom has written a text which gives a good succinct general description of different aspects of the way that a bird lives, beginning with a chapter on the physical characteristics of some of the more exciting birds of the world. This is followed by chapters on their evolution and a description of the families of birds, how they feed, their courtship and nesting, migration, and finally a chapter on the economics of birds as they are used by man or as pests. The colour photographs are excellent and as a general rule the legends are informative.

Penguin (Peter Owen: London, 1977; £5.95) is another seabird title, but the book is a personal account by L. Harrison Matthews apparently written from his 50-year-old diaries about a visit with the *Discovery* expedition of 1924 to the Antarctic, on which he was primarily engaged in whale research. There are six chapters, five of which are headed by the names of various penguins and one chapter entitled Captain James Cook. The text is a mixture of anecdotes of early travellers in the far south, personal reminiscences of his own life and voyages, and of the birds encountered on them. The ornithological interest is rather thin, comprising extracts from his diaries about the birds he met 50 years ago and occasionally referring to more up-to-date work. The photographs were apparently taken at the same time. More recently taken and clearer photographs might have helped the layman, for whom the book must have been intended, with the identification of the species referred to in the text. It is a pity that the author did not stick to the subtitle *Adventures among the Birds, Beasts and Whalers of the Far South* as the main title for, as a personal account of his travels in the far south, this is an amusing book to read. The title *Penguin* is misleading.

Whereas most of the books that I have been reviewing have been about the results of other people's research, there is only one book which described the techniques of watching birds. In this case, as its name *Bird Count* (Kestrel/Penguin: Harmondsworth, UK, 1977; hardback £2.75; paperback 75 pence) suggests, it summarises the various methods which have been developed largely by the British Trust for Ornithology for their surveys and censuses of the bird populations of various habitats in Britain, in which they have involved a large proportion of their membership. This book is intended for



Taken from *Birds of the Coast and Sea*

anyone interested in discovering more about wild birds in a methodical and thorough way. Humphrey Dobinson begins with a chapter identifying about 60 common birds, which I would have thought was slightly out of place; anyone who was going to use the techniques that followed would need to have had far more experience at identifying birds before embarking on the surveys. He follows with chapters on the various methods of counting birds. The common bird census

has been an invaluable technique in showing changes in the population levels of breeding birds since 1962. The technique was originally introduced at a time when many of Britain's birds were dying as a result of the misuse of the organochlorine pesticides and when it was felt that the bird population needed to be monitored on an annual basis. The author gives a summary of some of the results of the first ten years of the survey. Particularly noteworthy was the discovery that several species of birds which bred in this country were severely hit by the disastrous droughts in the Sahel region, and population levels of several species, notably whitethroat and sand martin, were considerably reduced. The count also shows that the populations of many wintering species, goldcrest amongst others, have soared in recent years because of the lack of severe winters. Dobinson then goes on to describe other techniques of counting birds on waterways, how to count colonial birds including seabirds, how to find nests, and how to study migration.

The book does not contain all the detail that a textbook would have, and anyone seriously needing to use these well-tried techniques would need to seek the original papers. There are also simpler methods of assessing the relative abundance of birds; this he does not mention. Mr Dobinson is a schoolmaster and his book was, I felt, written very much with the senior pupils and other amateur bird-watchers in mind, who may have passed beyond the listing and identification stage and want some project which will give them something more to think about. For this purpose, the techniques can produce useful results. Mr Dobinson has produced a very useful and serious book, which contains much useful information.

Peter Conder

Peter Conder recently retired as Director of the Royal Society for the Protection of Birds.

Bird-watching in West Africa

A Field Guide to the Birds of West Africa. By W. Serle and G. J. Morel. (Collins: London, 1977.) £5.95

THIS new *Field Guide* should prove indispensable to the growing number of bird watchers who visit West Africa. The area covered includes the entire bulge of Africa south of the Sahara and extending eastwards to Chad and the Central African Republic. The text is clear and concise, and contains many useful nuggets of information: how to tell, for instance, the difference between the African golden oriole and its European counterpart, both of which may be in the same area at certain times of the year.

The coloured illustrations are, of course, vital to amateur and professional ornithologists, and most of them are reasonably accurate, though a few are perhaps a shade too bright. It is a pity that the birds of prey are not in colour, as this group of birds is notoriously difficult and confusing to identify. The picture of the mouse-brown sunbird (opposite p225) shows a typical down-curved beak, whereas the description (p229) clearly states that this sun-bird has a short, straight beak.

These points aside, however, and bearing in mind that the book has around 1,000 birds to deal with, this *Field Guide* reaches the high standard which ornithologists all over the world have come to expect from Collins.

J. & G. Newmark

J. & G. Newmark have recently returned from an ornithological field excursion to West Africa.

All the world's a stage

Manwatching: A Field Guide to Human Behaviour. By Desmond Morris. (Jonathan Cape: London; Abrams: New York, 1977). £7.95; \$16.95.

"ALL animals", says Desmond Morris, "perform actions and most do little else". And all popularisers of ethology, he might have added, utter platitudes—and most do little else. The problem with his *Field Guide to Human Behaviour* is not so much that it's simplistic (which it is) or misleading (which it is) as that it's astonishingly trite.

It is trite and at the same time demeaning. If a zoologist is going to turn his binoculars—or opera glasses—on human beings he must get them the right way round; otherwise the people under study become manikins, the "field" of human behaviour becomes the land of counterpane, the Human Comedy a puppet show. Morris, here as never in his earlier books, comes over as an out-and-out reductionist. Animated little men and women trip across his stage, selfishly pursuing the goal of their own biological survival, pair-bonding and pair-breaking, staking out territory, hunting the Sunday lunch; they wave to each other with one of the three types of wave, they show their friendship with one of the 14 types of tie-sign; they have their exits and their entrances. Most of the time they use clothing signals to cover these exits and entrances from view.

Morris claims that as a "manwatcher" he is simply doing for people what bird-watchers do for birds: he records their behaviour and attempts without bias to provide historical or functional explanations for it. But when men watch birds they are observing a foreign species; they can assume no privileged knowledge of what the birds are doing or why. When men watch men, however, (as perhaps when birds watch birds) such an assumption of initial ignorance is at best tedious and at worst insulting to common sense. If a bird was to read this book, it might well find many of Morris's observations novel and intriguing. A person reading it, however, is certain to be less impressed: he must find much of the material boringly familiar (because he has been a manwatcher as long as Morris has), he must be irritated by the pseudo-scientific classification of behaviour patterns (because he guesses that they are largely Morris's invention), and he must realise that many of the biological explanations are plain silly (because he cannot believe, for example, that "viewed biologically, the modern footballer is revealed as a member of a disguised hunting pack").

That said, there are nonetheless places in the book where the old Morris whom we knew and (some of us) loved takes over. Hidden in the bran, there are a few real prizes. Yet even when Morris does say

something thought-provoking, the effect is lost because he fails to follow through. For reasons best known to himself and his editor, he quotes no authorities and no sources of evidence (the reading list at the back of the book has no immediate bearing on the text). The result is that all his more interesting assertions are left hanging in thin air. An informed reader may of course sometimes recognise where the ideas come from, but when he does so he will not always be reassured. For Morris is indiscriminating in what he is prepared to relay as established scientific fact: he has lifted some good ideas from the scientific literature, but he has lifted a lot of rubbish too.

If people get little from reading the book, they may still find pleasure in looking at it. It is beautifully and informatively illustrated. Indeed, the pictures often succeed where the words fail in giving insight into how human beings behave. Again and again, the evidence of particular photographs belies the stilted text. Here are real people fighting, loving, praying to God... The camera, framing a moment of their lives, calls us to attend to things which we half knew but never waited to consider. Meanwhile, Morris calls us to attention, tells us what to think: "Religious displays are essentially submissive gestures performed towards a super-dominant figure—the deity..." "More intimate than the Hand-in-Hand is the Waist Embrace (top



Female sporting behaviour

Taken from *Manwatching*

right). Because it involves close trunk contact and impedes locomotion, it is usually employed by strolling couples with time to spare..." "The butcher's shop has become the modern hunting ground, but now it is the woman who carries home the 'kill', while her male engages in symbolic hunting called 'work'". In his own words: "Some individuals find the wink difficult to perform even as adults".

Nick Humphrey

Nick Humphrey is Assistant Director of Research in the Sub-Department of Animal Behaviour at the University of Cambridge, UK.

Animal collections in the UK

Animals on View. By Anthony Smith. (Weidenfeld and Nicolson: London, 1977.) £6.50.

A GOOD guide should be concise, accurate and up-to-date, at least at the time of publication. Anthony Smith, in his extremely well illustrated guide to Britain's safari parks, zoos, aquariums and bird gardens, succeeds in achieving these requirements most successfully, and the general user will have little to complain about. The eyebrows of the regular zoo user may be raised a little over one or two caption errors which make parakeets of love birds and American bison from European ones.

It should not be imagined that the guide takes a clinical approach to the subject. Anthony Smith has done too many exciting things elsewhere to allow this to happen. He describes the factual details of the collections without turning it into a checklist of all the flora and fauna, but emphasises those aspects of breeding, exhibition and use for which individual zoos are famous. He also goes on to assess the management of some of the larger collections and in one or two cases he accurately predicts their closure. His assessments, which are of course the impressions gained on his visits to all the major collections in the guide, add another

interesting dimension to the work.

Anthony Smith's introduction to the book covers most aspects of animal collection management and the problems which can arise. I think he is very right to bring out these points, although one gets the feeling that here is the embryo of another book, which hopefully will be forthcoming, as the introduction is bound to leave very large question marks in many readers' minds.

His bias towards safari parks does shine through a little too brightly for the average "zoo man", but this is perhaps inevitable from someone who has been fortunate enough to spend much time in the wild places of the world and who is perhaps more aware of space in an ethereal sense than in terms of practical animal management. There are good zoos and good safari parks in terms of how much regard they give to the welfare of their animals. Sadly there are also bad ones in both categories.

The work is an important addition to any regular zoo user's bookshelf, and I believe that the more casual of zoos' twelve million or so annual visitors would obtain considerable interest and insight into the enormous variety of creatures that may be seen in collections, large and small, throughout the length and breadth of the UK.

R. J. Wheeler

R. J. Wheeler is Director of the Royal Zoological Society of Scotland, Edinburgh, UK.

African wildlife

PICTURE BOOKS about African wild animals suffer a population explosion inversely proportional to the decline in the populations of their subjects. All the arts and crafts of photography and book design combine in sharpening their competition for dominance in the face of the selective pressure of the bookshop browser, who is himself in danger of being bewildered by the plethora of jostling titles.

Some books are the outcome of scientific studies on the lives of various large animals, and are valuable contributions to knowledge made by naturalists who, surprisingly, seem now to prefer being called ecologists, ethologists, and so on, as a fancied boost to their dignity—Linnaeus, Gilbert White, Darwin, Wallace, Bates and the other giants of the past needed no such props.

Most of the books, however, are essentially picture books, often of the highest photographic standard, showing the beauties of tropical scenery and animal life. They concentrate on the eye-catching and spectacular parts of the flora and fauna, but give little information about them beyond the "visual display".

The third class is that of the doomsters bemoaning the fact that the frontiers of the wilderness are steadily receding in the face of ever-growing human occupation and development of the land. Much as the loss may be deplored, it is a fact that will not go away; wildlife is rapidly becoming confined to reserves and parks which, as time goes on, will become more and more artificial under management and conservation. Only those species such as the jackal in South Africa, and the fox and badger but not the otter in Great Britain, that can survive persecution by man, will remain as wild animals. Even now the average package tourist, conducted to viewing points, and sheltered in hotels or lodges with all European amenities so that he is protected from any close contact with the wild, sees little more than he does in a 'Safari Park' at home—only the scale is larger. The very word 'safari', used by the exploiters to beguile him with romantic overtones, merely means a caravan of beasts of burden, or a journey.

The White Lions of Timbavati, by Chris McBride (Paddington: London, 1977; £5.95) is the by-product of a scientific study on the lives of a group of lions that McBride kept under observation for about a year in a large private nature reserve in the north-eastern Transvaal. The group consisted of two dominant males sharing half a dozen lionesses accompanied by their cubs and adolescent offspring. One of the lionesses produced a couple of white, but not albino, cubs, on whose birth and early life the story concentrates. Later, another white cub was born to a different lioness, and the author thinks the white

births were a result of the frequent matings between father and daughter or granddaughter, so that homozygotes for the recessive white gene appeared. The white cubs were handicapped by their lack of concealing colouration, and probably survived only because McBride provided them with food at critical times. His book tells of his adventures with his wife and small daughter living in a bush camp while studying the lions, and makes an excellent read. He has a pleasant straightforward style of writing, and in addition to his narrative he sets out his common-sense thoughts on wildlife conservation and management.

Savage Paradise, by Hugo van Lawick (Collins: London, 1977; £13.95), is a picture book. Van Lawick claims to be a



Lions

cameraman first and only secondarily a naturalist. His large coloured photographs, up to 20 by 14 inches in size, illustrating the lives of the predatory animals of the Serengeti plains and their prey, are unsurpassed in subject and composition. The author hopes that his pictures will reflect his love for the Serengeti and the Ngorongoro Crater; "but in an honest way, for with its beauty there is also harshness, a savage struggle to survive in a paradise." *Savage* indeed is the picture of half a dozen hyaenas tearing out the entrails of a living zebra; in spite of the zebra's facial expression, the author assures us that "it is doubtful if the prey feels much pain", a thought that may comfort him if he ever finds himself in a similar predicament. He prefaces his book with informative essays on each of his subjects—lions, leopards, cheetahs, hyaenas, jackals, and wild dogs—detailing his experiences with each species and "some of the remarkable things that happened while I was watching them." This is a superb book of its class.

Pyramids of Life, by John Reader and Harvey Croze (Collins: London; Leppincott: Philadelphia; £6.95; \$12.95), is a very different book. Although every page is adorned with splendid photographs taken by Reader, the pictures are chosen to illustrate the text of each 'double spread' by Croze. The main theme of the book is the circular movement of energy through each

ecosystem, the cycle starting with the soil receiving sunlight, water and air to produce plants that are eaten by herbivores which in turn are eaten by the carnivores at the top of the pyramid. At any point in the ascent from primary producer to carnivore, the organisms may die, whereupon their bodies are reduced by mostly microscopic decomposers into simpler substances which return to the soil for the start of another cycle. All this may seem obvious enough, but the infinitely varied paths that the cycle can take, and the inter-relationships of the plants and animals concerned, as described and pictured here, will be a revelation to the intelligent layman who prefers to understand something of what he sees rather than merely making it the subject of holiday snapshots. In the epilogue, Croze points out how knowledge of the way the amounts of energy and materials determine the number of animal and plant pipelines, up through the pyramid of life and back down to the soil, can be used to preserve the richness and yield of terrestrial ecosystems; and that we should not be discouraged by the notion that natural beauty is not its own excuse for existence. More care in editing the book would have avoided such a solecism as 'an algae is', repeated twice.

In Noah's Ark is Stranded, by Björn Berglund (Macdonald and Jane's, 1977; £6.95), the author claims to deliver the "message of African ecology". Berglund is a Swedish journalist who wrote his book after visiting nine East African national parks, where he was shown all the usual sights. The accuracy of his observations on animals may be judged by the double-page photograph of a crocodile walking in a manner exactly the opposite to that stated in the caption. The author seems to suffer from the typical Swedish guilty conscience, for throughout his book he gives the main emphasis to bemoaning man's destructive impact on his environment in Africa. He tells us that "ecology teaches the dependence of the living organism upon the environment and their harmonious interaction", and concludes with a glimpse of the obvious in the admonition that "we must accept that we function within an environment", asking: "are we able to live in harmony with each other and our environment?". He need not look far for the answer.

Back in the Wild, by Sue Hart (Collins: London, 1977; £5.50), is a collection of short articles reprinted from a South African newspaper. Most of them are about conducted visits to various parks and reserves, where the author seems to have looked on the larger members of the fauna as people dressed up in animal skins. The articles are so slight, superficial, and often inaccurate as to fact, that one well may wonder why a publisher should bother to reprint them. The drawings by Mrs Leigh Voigt are better than the text.

L. Harrison Matthews

L. Harrison Matthews was Scientific Director of the Zoological Society of London from 1952–66.

Ornamental creatures

CHRISTOPHER LEVER's exciting and scholarly book *The Naturalized Animals of the British Isles* (Hutchinson: London; £7.50) is full of surprises. Did you know that there are Mongolian gerbils on the Isle of Wight, Midwife toads in Bedford, Pumpkinseeds (a species of sunfish) near Tunbridge Wells and Wallabies in Yorkshire? These strange animals and many more are established in the wild in Britain in self-maintaining populations. Many of our more familiar animals have also been introduced by man, including the rabbit, brown rat, house mouse and grey squirrel. Fifty-nine species of vertebrates are described with a separate chapter devoted to each, in which the author describes exactly how, from where and when, the animal was introduced, and summarises its biology and the effects of the introduction on our native flora and fauna.

Many species, such as the pheasant, were introduced on purpose for their food value. Others were brought here for "economic reasons", like the little owl, imported from Italy by an eccentric who had the inspired idea that it would be a useful means of pest control in the kitchen garden and would also rid our church belfries of their sparrows and bats. The difficult question of the advantages and disadvantages of introducing foreign creatures is discussed. Few would deny that we would be better off without the rat; but for Christopher Lever, at least, life in Britain would be miserable without such ornamental creatures as the beautiful and harmless Mandarin duck, to whom he dedicates his fascinating book "with admiration and affection".

Introduced animals, however, can have disastrous effects. Feral dogs on the Galapagos Islands have gone wild and are decimating several of the island populations of land iguanas. A captive breeding project has been set up in an attempt to protect these magnificent beasts. This, together with many other conservation efforts by the World Wildlife Fund (WWF), is described in their yearbook *The World of Wildlife* edited by Nigel Sitwell (Hamlyn: London; £2.95). The book, which is illustrated with some superb photographs, contains sixteen chapters on endangered species, including the tiger, narwhal, whooping crane and desert pupfish, and describes the efforts that are being made to save them and their habitats. Thanks to the successful Operation Tiger campaign, launched by the WWF, reserves have been set up throughout India, and the tiger now has a good chance of survival.

The most dramatic success of the WWF conservation effort last year must be the Italian government's agreement to give

complete protection to the hundred or so remaining wolves in the Apennine mountains. And let us give a thought this Christmas to the beautiful scimitar horned oryx, now in grave danger of extinction. An important breeding centre has been set up in the Marwell Zoological Park in Hampshire, so perhaps we can look forward to the day that the oryx join the wallabies as naturalised British citizens?

I sometimes wonder just why it takes so long for new ideas in the scientific literature to filter through to the popular book market. *Inside the Animal World* by Maurice and Robert Burton (Macmillan: London; £6.95) is subtitled *An Encyclopaedia of Animal Behaviour*, and although it provides an extensive catalogue of behaviour, it leaves little room for explanations of how the behaviours could have evolved or what ecological circumstances favour certain behaviour patterns rather than others. Thus, we are told that some species of frogs are good parents though others are not; but the interesting question is why is there a difference?

The vital function of territorial behaviour is said to be the regulation of population numbers so as to prevent overcrowding. But I feel that the layman is entitled to an answer to the question of why the "doomed surplus" accept their miserable role in life. There is next to no reference to the fact that different individuals may be selected during evolution in different ways. How much more fascinating courtship behaviour becomes

when we realise that optimal reproductive strategies for males and females are rarely similar. Animals may often signal their sex "because it is a waste of time for a male to court another male", but in some instances it apparently pays a male to mimic female behaviour. Some male elephant seals pretend to be females (the "Danny la Rue strategy") and join a harem to steal copulations, unnoticed by the dominant male. Surely we can no longer be satisfied with explanations of courtship displays as "designed to overcome the fear of close contact", or of lions killing young fathered by other males as a result of a "feeding frenzy". Male lions can in fact enhance their reproductive success by such murderous behaviour, because this brings the females into oestrus again sooner and thus hastens the day that the male can sire his own offspring.

This is, however, an attractive book and will fascinate those readers who want to learn about how wonderful nature is, without wanting to know the reasons why. I feel that the lovely line drawings by Hilary Burn and the outstanding photographs by Jane Burton deserve considerably more acknowledgement than their brief mention on the flyleaf of the dustjacket.

Nicholas Davies

Nicholas Davies is Demonstrator in Zoology at the Edward Grey Institute of Ornithology, Department of Zoology, University of Oxford, UK.

Desert habitats

IN spite of all the constraints desert conditions impose on human activity, they do generate in the individual a great sense of freedom. This, perhaps, explains why so many of those whose names are so closely linked with arid regions were, above all, great individualists. Arabian explorers like Lawrence, Philby and Thesiger have their counterparts in the New World—naturalists who, in their very different way, were no less individualistic and devoted to the desert habitat. Twenty years ago, two books of Edmund C. Jaeger (now in his nineties, and still enjoying the desert environment in Riverside, California) formed an excellent introduction to the North American deserts, particularly those of California. In the early sixties, Alonzo Pond wrote his highly individualistic account of deserts in *The Desert World*. In the same mould is Raymond B. Cowles. Born and raised in the South African bush, he subsequently spent nearly sixty years studying the adaptation of living organisms to desert conditions in Southern California. He pioneered work on reptilian thermoregulation; and his *Desert Journal*

(University of California Press: Berkeley, Los Angeles and London, 1977; \$10.95; £8.25) is a reflective account of the background to these studies. The work conveys his enthusiasm, insight and excitement at unravelling the nature of the behavioural and physical adaptations of desert animals to the lethal heat and aridity of their habitat. More than this, it gives the reader an appreciation of the total desert environment.

The Desert, by J. L. Cloudsley-Thompson (Orbis: London, 1977; £4.95), makes equally easy reading but is quite a different work. Connoisseurs of coffee-table culture may be forgiven if they recall similar offerings from other publishers (Time-Life, Aldus), some with the same title, some with similar photographic credits, some even with the same author. But readers can be assured that the text is different. Also, only in the volume under review are the cold deserts of polar regions considered in addition to hot deserts. At the price, this handsome volume is good value for the layman and widens his choice of introductory books from what must now be an almost saturated market.

M. J. Chadwick

M. J. Chadwick is Senior Lecturer in Biology at the University of York, UK.

Pictorial delights in marine science

THIS year has seen the publication of five books on various aspects of life in the seas and oceans. All are published to a high standard and are sometimes called 'coffee-table books', designed to attract the casual looker rather than the hard reader. All of them are intelligible to the non-scientist but some would be useful in sixth-form (advanced school) libraries or for general reading by undergraduates, thus giving a wide potential market.

Probably the most information is packed into *The Undersea* (Cassell: London; Macmillan: New York; £12.50; \$27.50), a book of grand dimensions edited by N. C. Fleming of the UK Institute of Oceanographic Sciences. It contains chapters by different authors on the ocean floor, the water itself, plant life, salt-water animals, the ocean resources, the use of ocean space, underwater archaeology, diving and divers, submarine craft, and marine law and politics. This is oceanology rather than oceanography. The book is expensive but the standard of production is high, with hundreds of black-and-white and coloured photographs and diagrams. A major criticism is that the legends for these are often inadequate and some of the diagrams are poorly conceived. Although some of the chapters are difficult, for example that on the ocean floor, most of them will be understood by the lay reader. There is a good index and modest bibliography.

The other four recent books are more limited in scope. In *The Seashore and Its Wildlife* (Orbis: London; £5.95), Robert Burton, a naturalist author, sets out to describe the nature of the shore, how it is formed, its variability in different parts of the world, and its flora and fauna. This delightful book is packed with excellent colour photographs, and the text is readable and informative, with a good index and bibliography. It must rate as a best buy in terms of production standard and price, if one wants a book on the shore, but I would rate it educationally only up to sixth-form level as general reading.

In a rather similar category is *Animals of the Oceans: The Ecology of Marine Life* by Martin Angel and Tegwyn Harris (Eurobook/Peter Lowe: London; £4.75), which contains many excellent photographs but also some poor and distinctly garish diagrams. There are chapters on the ocean environment, the ocean's fishes, the edge of the sea, coral reefs and mangrove swamps, plankton, the open sea, air-breathers, and man and the ocean. There is a short bibliography and glossary, and an index. This book is obviously good value for money; it is elementary in style but readable and

fairly informative. Perhaps the authors tried to do too much in the space available.

Finally, there are two books on marine mammals. In *The Encyclopaedia of Sea Mammals* (Hart-Davis, MacGibbon: London; £12) D. J. Coffey sets out to produce something approaching an encyclopaedia. There are three main sections: dolphins, whales and porpoises; seals, sea lions and walruses; and dugongs, sea cows and manatees. With each group there is an alphabetically arranged list of features on life history, ecology, exploitation, and so on, followed by a list of species, each briefly described. There are some indifferent diagrams, some really

identification. Both books bring out well the lore surrounding sea mammals, especially the Cetaceans, and they rightly point to the problems of conservation. I find their strictures on the use of dolphins in warfare less easy to understand. It seems to me that using dolphins to flush out enemy frogmen or carry limpet mines is little different from the widely accepted use of police dogs or horses.

When reviewing popular or semi-popular books of such a high standard of production, an inevitable comparison is made with the strictly technical literature, where £20-30 is a common price range.



The capture of a whale is seen as cause for general excitement in this Dutch sixteenth-century print. The workers' efforts are spurred on by the bagpipe player and flagbearer (left), while in the background less fortunate whalers fall prey to fearsome monsters. Illustration taken from *The Encyclopaedia of Sea Mammals*, reviewed on this page.

excellent photographs, and copies of prints and other pictures dealing with the subject. The academic standard is about sixth-form level. Although the book could be used to identify some of the species described, it is by no means a key to identification, and many of the photographs are taken for dramatic effect rather than recognition. At £12, this book is by far the highest-priced for its size. Perhaps the price is set high because the book is of more limited interest and the potential sales therefore lower.

Mammals of the Seas by R. M. Martin (Batsford: London; £4.95) is a book of similar length, style and standard to the Coffey book. The difference in price, however, is astonishing. Species are listed and there is a glossary, index and bibliography. It is a pity that neither book contains a really good key or section on

Presumably, such technical books sell 1000-5000 copies. What is the estimated sale for the books under review? The publishers no doubt have done their market research and are exploiting the present vogue in natural history and the oceans. Sales will go up before Christmas, since such books will catch the eye of parents seeking presents; these books may get into school and public libraries, and individuals may indulge themselves. As a marine biologist, I like *The Undersea* best. All the books, however, contain an excellent range of colour photographs, and even if the text of some of these books is banal in places they are a pictorial delight.

J. H. S. Blaxter

J. H. S. Blaxter is Senior Principal Scientific Officer at the Dunstaffnage Marine Research Laboratory, Oban, Argyll, UK.

Medicinal and herbal plants

PUBLISHERS seem to be producing an army of books on the power of plants—how to grow them and use their products. Here are two more popular texts for those inclined to do-it-yourself medication or those interested in the subject from a more academic angle.

Guide to Medicinal Plants by P. Schauenberg and F. Paris (Lutterworth: Guildford, UK, 1977; £5.95) is translated from the French and is a straightforward, workman-like production. It is responsible in its attitude towards a subject which is not always treated with the respect it deserves: there may be a thin line between therapeutic and poisonous doses in some medicines of plant origin.

The plants are arranged according to their active principles—alkaloids, flavonoids, saponosides, tannins, and so on—each chapter being prefaced by a short

compresses makes fascinating reading: a gargle based on sage and red wine might make a sore throat almost worthwhile.

The occasional line drawings scattered through the text are not very helpful and could well have been omitted, but the coloured drawings at the end, though not outstanding, are pleasant and the plants recognisable.

A History of Herbal Plants by R. Le Strange (Angus and Robertson: Lewes, UK, 1977; £8) is in complete contrast. The author has obviously read widely, if somewhat indiscriminately, and has, it seems, put down as much about each plant as his publisher would allow. Unfortunately, not all the information is accurate, and the nomenclature, both Latin and English, leaves something to be

desired—the Oregon grape has been known as *Mahonia aquifolium* for very many years now—but this no doubt is due to inadequate sources of reference.

Each entry is illustrated but the drawings are often rather odd and in a number of styles; some, such as that of *Viola tricolor*, resemble those in Victorian gardening books and are charming, but some of the others are formalised to the extent of looking like decorative tiles, as in the *Aristolochia clematitis*. *A History of Herbal Plants* is, however, quite entertaining.

Rosemary Angel

Rosemary Angel is Head of the Museums Department at the Royal Botanic Gardens, Kew, UK.

Making use of twigs

Plants with a Purpose. By Richard Mabey Pp. 176. (Collins: London, 1977.) £4.50.

RICHARD MABEY wants us to recapture the sense of intimacy with plants that was known to our ancestors, and to help us he has written a book about the household uses of some of the commoner wild plants of Europe and North America. The result is a hybrid between a historical survey and a collection of helpful hints, which at first glance might seem appropriately subtitled *A Hundred and One Things to do with Twigs*.

The versatile twig and its close relatives, the stick and the sprig, certainly feature prominently, and it is possible to imagine a culture based on their use. In the dark ages, before science and technology began, the twig people would rise early from their heather mattresses, and chew on twigs to clean their teeth. They used bundles of birchwood to light their fires, on which they cooked a mess of pottage prepared using whisks made from handfuls of birch twigs—very satisfying to use, though rather fiddlesome to wash, Mabey assures us. They swept

their floors with birch brooms and strewed them with pine twigs. Each day would include an expedition to the forest to collect twigs. Older twig people took their ash walking sticks, and everybody wore sprigs of elder or wormwood in their hats to keep flies at bay. The day's collection of woody material was carried home in wicker baskets woven from willow twigs. When a woodland fire was encountered, it was beaten out using a bunch of fine birch twigs—presumably larger than those used for the whisks. The twig children usually played with catapults made from small forked sticks.

It seems unlikely that this book will stimulate a renaissance of the twig culture, or start a mass vogue for rush-lighting, nut polishes or homemade herb shampoos. Doubtless, the Forestry Commission would not be overjoyed if it did. But a *pot pourri* or a pressed-leaf bookmark is a simple reminder of the versatility of plants. Richard Mabey's book includes many such reminders, and will encourage a greater appreciation of the value of plants. Perhaps it will even help to restore their former intimacy.

Mary Lindley

Mary Lindley is Assistant Editor of *Nature* and a former student of botany.

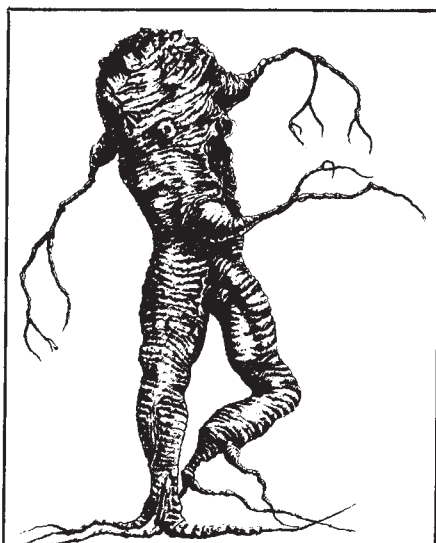


Illustration of *Bryonia dioica* (root), taken from *A History of Herbal Plants*, reviewed on this page. In the fifteenth and sixteenth centuries, the roots of the Briony were roughly shaped to represent the human form and implanted with Millet sprouts, to simulate the hairy Mandrake. These were then sold for large sums of money because of their reputed medicinal powers.

account of the constituents under consideration. Most of the plants listed are either native to or commonly cultivated in Europe, and at the end of the book there is a chapter on medicinal plants from other continents. This latter seems to be a fairly arbitrary selection, varying from coffee and sarsaparilla to nux-vomica. The entry for each plant is short but adequate, giving Latin, French, German and English names, distribution, a short description, flowering and collecting seasons, active constituents, properties, and so on. A chapter on tisanes and

Victorian Natural History

The Family Naturalist. By Michael Chinery. Pp. 192 (Macdonald and Jane's: London, 1977.) £6.95.

I WAS fortunate enough to have a mother who was born in 1873, when Queen Victoria was on the throne, and who was one of the earliest women students of biology at what was then the Durham College of Science in Newcastle upon Tyne. Marriage prevented her from exercising her pro-

fession, but she introduced me to Natural History. From the age of three, we collected wild flowers, probably illegally, in the untidier parts of the Pollock Estate in suburban Glasgow. When, at the age of seven, I moved to rural Renfrewshire, I could already identify many of the common plants and insects. I pressed flowers and reared caterpillars in the nursery, and scared the maids with newts which escaped and found their way into the kitchen. We had all the texts for the well-brought up child. I remember particularly *Eyes and No Eyes*; this and other books of the same genre deserve to be reprinted.

This interest in Natural History was not as elitist as some may think today. My earliest mentor, outside the family, was David Borland, one of the few surviving handloom weavers in the village of Kilbarchan. He was a rugged independant, enjoying a penurious self-employment which gave him freedom to take time off and wander the countryside with two or three boys, who were constantly fascinated by his knowledge of wildlife and his powers of observation. I continued to be lucky when I went away to school, as on half-holidays I was allowed to explore the Teesdale countryside, occasionally guided by Bentley Beetham, who later graduated to Everest expeditions, from climbing cliffs to photograph peregrines.

Children today are not so fortunate. They do indeed have a marvellous selection of glossy Natural History books, lavishly illustrated, but few of these contain the sort of "meat" so satisfying to the enquiring child, that the improving Victorian writers and teachers provided. Michael Chinnery's *The Family Naturalist* fills this gap. It explains how to record, to observe and to investigate all aspects of Natural History. It is written simply enough to be understood by quite young children—at seven, I would have made it my bible. Even parents with no previous knowledge of the subject will be able to follow the text and the instructions. At the same time, professional ecologists who find it difficult to communicate their interests to their own children will find it invaluable—it may even enlighten them on some aspects of their own speciality. Although Michael Chinnery adopts the approach of the best Victorian naturalists, he is also uncompromisingly contemporary. He realises the need for conservation and the danger the ardent collecting habits of the nineteenth century naturalists would present if they were adopted by thousands of present-day children, when pressures on our flora and fauna are so much greater.

The book is therefore warmly recommended to both parents and children, whether or not they are already actively involved with Nature. Even town dwellers who only visit the country occasionally will find how to make those visits more interesting, and learn what (and what not) to take home for further study. Some of the exercises can even be carried out in urban areas.

Even this excellent book, however, will not work miracles. Some parents will be disappointed if they find they have children with no in-born interest in Natural History. My elder brother, brought up in the same environment as I was, never became remotely interested in plants or animals. But there are a great many parents and children to whom Michael Chinnery's splendid book will bring great pleasure and a much better understanding of the world in which they live.

Kenneth Mellanby

Kenneth Mellanby has been Director of Monks Mood Experimental Station, Abbots Ripton, UK.

A talent to amuse

Worlds within Worlds: A Journey into the Unknown. By M. Marten, J. Chesterman, J. May and J. Trux. Pp. 208. (Secker and Warburg: London, 1977.) Hardback £7.95; paperback £3.95.

PERHAPS the least effective method of obtaining funds for a project is to argue that the results will provide pleasure for idle people. But when more telling arguments have extracted the money, the technology has been developed, and the results have appeared, then the pleasure may be there all the same and we would be foolish not to take advantage. That we can do with *Worlds within Worlds*, a collection of pictures resulting from advanced technology that allows us to be entertained during moments of idleness.

Not all such collections have the ability to entertain. The authors of this one are therefore to be congratulated on their choice of material. In a book of some 200 pages, they present a sample of images covering the wavelength range from audio to X rays. It has been done before—indeed, it has frequently been overdone, and certain topics have appeared with a sickly regularity—but *Worlds within Worlds* provides a refreshing change. The

authors present an intelligent selection of pictures, including many old favourites: bullets bursting balloons, diffraction patterns, crystal structures, marine organisms, bacteria, and images of our internal organs. Satellite cameras produce fascinating views of the Earth and its weather; and the Solar System is the source of a variety of stunning photographs. But the most fascinating photographs are of the effects produced by invisible objects—heat images. False-colour images from computer coding provide a contrast with the realism of optical wavelength photography; and the authors are sensible enough to include a judicious selection of black-and-white photographs. The result is a book of surprises and contrasts with great visual appeal.

The same intelligence can be detected at work in the figure captions, but with less success. It is unfortunately a luxury to expect information from the text of a book of this type; yet, here the pictures have been chosen not only for aesthetic reasons but also because there is a story to tell. Often the captions are interesting and informative, occasionally fascinating, but sometimes they degenerate into the naive and sensational. Although below the high standard of the pictures, the text nevertheless adds to the enjoyment of the book rather than being an irritant. Relating the text to the pictures, however, can be frustrating: faint numbers can be seen below the pictures

Colorado River Delta in the Gulf of California. The River is the black expanse at the bottom of the picture, with its waters branching out through the white sandbars to gain access to the sea at the top. Illustration taken from Worlds within Worlds, reviewed on this page.



after some hard searching, but they could usefully have been more obvious while remaining unobtrusive.

A brief guide to the electromagnetic spectrum, and the techniques used to explore phenomena at different wavelengths, rounds off the book. This is not a reference book and not a book

to be read, but it is more than a coffee-table book designed to impress. An intelligent choice of pictures and words makes this a volume that will fascinate and inspire. It is well worth the asking price.

Stuart Sharrock

Stuart Sharrock is Physical Sciences Editor of Nature.

Palaeontological pop

THE Christmas dinosaur season is upon us once again. Two major offerings of large format by Richard Moody and Michael Tweedie both have authoritative texts. Moody's *Natural History of Dinosaurs* (Hamlyn: London; £2.95) has a 29-page introduction that is packed with information, highly condensed and is by no means an easy read. Evolutionary changes are documented but for my money I would have liked to have seen a more extended discussion of the functional aspects, and at least something on the other aspects of the contemporary faunas to give a more rounded picture of the natural history of dinosaurs. There are one or two jarring bits of information. I have never heard of the pelycosaur *Dimetrodon* having its teeth "differentiated into incisors, canines and cheek teeth" and the occasional purple passage such as certain dinosaurs "grinding mouthfuls of 'Mesozoic cud'" have implications with regard to feeding habits that are unfounded. But really I have no quarrel with the author's contribution. It is a worthwhile job well done, given the limited space at his disposal.

The main part of Moody's book is a series of plates with explanatory accompanying texts. The type-face is larger, and the mini-articles are easy to read and surprisingly informative; again the author has done a good job. This book is clearly intended to sell as a picture book, and five different artists have contributed to this. Ann Baum is a most accomplished artist and her illustration of *Acanthopholis* playing wheelbarrows is far and away my favourite dinosaur illustration. It is a charming picture and leaves one with the impression that the range of dinosaur behaviour was more varied than we had previously imagined. My next favourite is Tony Morris's duckbilled dinosaur *Brachylophosaurus* shown in a tender embrace of what could only have been the last waltz. A few illustrations are indescribably bad and grossly inaccurate. Finally it is a great shame that there were only two illustrations by Thomas Crosby-Smith. His *Mandasuchus* feeding on the corpse of a dicynodont in the pouring rain is magnificent. In conclusion, as a picture book, this is very much a curate's egg (good in parts) and I feel that the excellence of the text has not been adequately matched by the artwork.

The World of Dinosaurs (Weidenfeld and Nicolson: London; Morrow; New York; £4.95; \$14.95) by Michael Tweedie is clearly the work of an extremely accomplished writer. It is easy to read and gets across a considerable amount of information. Yet, on reading it, one gets a feeling of *déjà vu*. I suppose Alan Charig and friends should be flattered, but it is clear, at least to my colleagues, that this book is obviously derivative. One should not carp about this too much, for it is done with flair and in itself is an excellent account of dinosaurs. The running text is well illustrated, but interspersed at intervals are large coloured double-page spreads, a few are grossly inaccurate—for example, the ceratopsians the sauropods and the marine short-necked plesiosaurs. Other illustrations have a familiar ring and I am reminded of Giovanni Caselli proudly claiming that other artists would henceforth use his work as reference material; I am certain he will be suitably gratified to find his prophecy fulfilled.

The last two books are on fossils in general, and in the main are on the kind that anyone can hope to find. Rhona Black's *The Observer's Book of Fossils* (Frederick Warne: London; £1.10) is a neat pocket-sized book of modest price, which, after a concise introduction dealing with many aspects of palaeontology, gives a systematic run through of the major groups of fossils with paragraphs on the commoner forms. The line illustrations are perfectly adequate, al-

though their positioning at the bottom of each page gives the impression that they were an afterthought popped in just to fill up the page. The complete absence of an index is infuriating and must reduce the utility of this book enormously. The only other criticism I have is in the section on fossil collecting. My own preference is that notebook and pencil and wrapping paper are the prime requirements. So much unnecessary damage is caused at fossil localities by the over-enthusiastic use of hammers and chisels that their use should be positively discouraged; they are hardly ever necessary and do more harm than good. If fossils cannot be collected by hand, they should be left where they are.

With the final book on fossils, we are back to the Christmas market. Richard Moody's *The Fossil World* (Hamlyn: London; £2.95) is a glossy picture book with attractive colour photographs of fossils. Instead of trundling through one fossil group after another, Moody has dealt with the three major fossiliferous eras: the Palaeozoic (43pp), the Mesozoic (23pp) and the Caenozoic (17pp). The text is of a high standard and reading through it one gains a vivid impression of the changing fortunes of animal life through geological time. Again, I would have preferred rather more on the functioning and behaviour of the organisms he describes. Lots of technical terms suddenly pop up without any explanation and I personally found the sections dealing with bivalves and echinoderms particularly hard going. It was a surprise that the coccoliths as the major component of the Chalk rated not a mention in the Mesozoic section. Basically, I liked this book very much and I hope it does well.

Beverly Halstead

L. B. Halstead is Reader in Geology and Zoology at the University of Reading, UK.

Megalithic architecture

The Megalith Builders. By Euan MacKie. Pp. 208. (Phaidon: Oxford, 1977.) Paperback £4.95.

THIS is a popular book, without references in the text either to sources or to the numerous photographic illustrations, which are of varying quality. The colour-plate of Stonehenge (p17) and the aerial photo (p77) and the interior colour view (p88) of the West Kennet Long Barrow are laterally reversed. The colour plate on p190 and the cover, ostensibly of Stonehenge near sunset in winter, is a clever photomontage, visually attractive but astronomically impossible, and should have been identified as such. Several of the black-and-white plates are dark, muddy or out of focus.

The author's declared aim is to "explain" the megalithic monuments of western Europe in their current chronological contexts. Formerly, it was supposed that megalithic architecture was diffused gradually from the Mediterranean around the western and northern coasts of Europe; but latterly corrected radiocarbon dates (and a series of thermoluminescent dates from Portugal with wide confidence limits, to be used with more caution than the author shows) have tended to imply that the earliest tombs were built on the Atlantic littoral. This is inconsistent with the older model of unidirectional diffusion from Mediterranean or Near Eastern sources of cultural innovation.

The scene is set by eight rather disjointed chapters on regional groups of sites. A surprising inclusion at the beginning is Skara Brae in Orkney, a

block of late Neolithic one-room flatlets hardly to be described as megalithic. An even more surprising omission is any treatment of the abounding megalithic tombs of the Netherlands, north Germany and southern Scandinavia. Thus, the descriptive foundation of the book is at best partial. It also contains avoidable errors.

The interpretation begins in chapter nine with an interesting examination of the nature and limitations of explanation in prehistory, which deserves close but critical attention. Mackie rightly says that "it is impossible to proceed directly from the archaeological evidence to the detailed reconstruction of the vanished societies which produced it"; but he believes that social explanations can be provided "by analogy—by looking at the known primitive peoples who possess a material culture similar to the prehistoric one" (and what about a similar environment?). There follows an imaginative discussion of alternative mechanisms of cultural change, modelled by analogy on theories of biological evolution. The Lamarkian, anti-diffusionist, model allows cultural or technological innovation to arise spontaneously (and maybe more than once in different times and places) in response to the stimuli of local conditions, natural and human. By contrast, the Darwinian model requires that an initial cultural mutation spreads ever wider from its source by a process of successive out-breeding and hybridisation.

On this basis, one would have thought that Gordon Childe, the chief architect of the diffusionist hypothesis in European prehistory, would have been classed as a neo-Darwinian. Surprisingly, however, MacKie sees him as a cultural Lamarkian, quoting these words from Childe's *Social Evolution*: "Inventions can be transmitted from one society to another, and that is precisely what diffusion means. But that is just what is impossible in organic evolution".

When this was written in 1951, it was believed to be true; but subsequent advances in molecular biology have shown that innovations can be transferred from one group of micro-organisms to another by genetic recombination. In this sense Childe's model of cultural diffusion was even more closely neo-Darwinian than he himself could have admitted. MacKie's analogy here is all the more strange, in view of his firm support for the model of social evolution put forward by C. D. Darlington in *The Evolution of Man and Society* (1968), in which cultural and genetic out-breeding reinforce each other through a synergistic

hybrid vigour, with the corollary that in both fields continued in-breeding leads to stagnation, etiolation and ultimate extinction.

The rest of the book is an attempt to explain the long and diverse development of European megaliths in Darlingtonian terms, as the result of the cultural and genetic impact on various neolithic societies of a class of far-voyaging astronomer-priests, itself the product of the expansive hybrid vigour of the early urban societies of the Near East. Here once more are Childe's "megalithic saints", though more exotically attired.

This is an interesting thesis, but its foundations are dangerously insecure. "That professional priesthoods existed in north-west Europe in the middle of the third millennium B.C. . . .", says MacKie, "is abundantly testified to by the stone circles and the inhabited earthworks and stone villages of late Neolithic Britain". But this is not testimony. The evidence itself compels no such conclusion, nor indeed any specific conclusion, because it is material, text-free and dumb. It has to be questioned, and the questioner supplies the answers himself. It is all too easy (and that is why the temptation should be resisted) to mistake an attractive but arbitrary speculation for an inference which the evidence itself imposes.

Amateur star-gazing

THE pace of astronomical and space research, the speed with which technological developments in optical and radio astronomy are implemented, the inventiveness of the astronomical mind and the productivity of the astronomy book publishers never cease to amaze me. This year, like others, the bookshops bulge with new titles and reprints of old favourites all eagerly awaiting buyers of all ages. This review considers ten books that are ready to be snatched up by the present-seeker as the festive season nears.

Let us start in space. *Flight to Mercury* (Columbia University: New York and Guildford, UK; \$16.20) is a diary of the Mariner 10 mission to Venus and Mercury, compiled by Bruce Murray, the director of the Jet Propulsion Laboratory of the Californian Institute of Technology and Eric Burgess, the well-known author of many technical and popular articles on upper atmospheric physics, rockets, missiles and space flight. Mariner 10 was launched in November 1973. Because of the economic recession at that time, this mission set new standards of cost control and efficiency. It was Man's first detailed look at Mercury, the innermost planet.

Although it is true that much archaeological explanation does rest on analogy (the author rightly says that we call a particular bronze artefact a spearhead because it resembles artefacts used as spearheads today), the ambiguity of identification by analogy increases very rapidly as we move away from technology to social structure and religion. In these fields analogy can explain little or nothing about the prehistoric past. It can do no more than enlarge the field of conjecture.

Furthermore, it is worth asking whether much of the "megalithic problem" is not really a non-problem. By definition, a megalithic tomb or temple is built of large stones. It has been assumed for centuries, and still is, that most if not all of the prehistoric megalithic constructions of Europe are manifestations, however diverse, of a common basic phenomenon, and require a unifying hypothesis to explain them. But why? No-one seeks a corresponding single hypothesis to explain the distribution, diversity and chronology of contemporary constructions of small stones, or for that matter of timber. May we not be searching in the dark for the black cat that isn't there? **R. J. C. Atkinson**

R. J. C. Atkinson is Professor of Archaeology at University College, Cardiff, UK.

It produced thousands of striking photographs of Mercury and Venus, over 100 of which are beautifully reproduced in this book. The book is riveting; reading it, you feel as if you are there, at mission control, living through the problems and triumphs with the members of the science and engineering teams. I didn't realise before just how many things go wrong with "successful" missions: television heaters don't come one, antenna power unexplainedly drops, manoeuvring gas nearly runs out, instrument bays heat up, bogus satellites of Mercury are discovered. The book is a space-fact adventure story and I recommend it highly.

From space fact to space fiction. *Colonies in Space* (Stackpole: Harrisburg, Pennsylvania; Van Nostrand Reinhold: Wokingham, UK; £9.85) by T. A. Heppenheimer, is just that (at the present time); but who is going to be dogmatic enough to say it could not happen. Heppenheimer shoots us forward to the days of space colonisation, tens of thousands of people living in attractive Earth-like space communities. We read of space farms, closed-cycle ecosystems, Moon Miners, asteroid tugs, orbiting power satellites, low gravity swimming pools, and interstellar flight. We are shown beautiful paintings of the interior of a space colony, most of these from the

brush of Donald E. Davis, a NASA artist. Heppenheimer is a planetary scientist, and in this well written book he draws on many serious and careful studies that show that Man has the scientific and engineering capability to put human life permanently into space. In his words, this "is no less than a major new stage in human evolution, the first step to star trekking across the light years of our universe". Heppenheimer paints a Eutopia in the sky but seems rather shy of counting the cost to those left behind on Earth. I'm left with the sneaking feeling that it would be easier to make a 'heaven on Earth' first.

For the Earthbound telescopicist, *Moon, Mars and Venus, A Concise Guide in Colour* (Hamlyn: London; £1.50) by Antonin Rükl, is an excellent buy. It contains a brief summary of Man's exploration of the lunar near side, a discussion of the height and formation of lunar features, the views of Venus and Mars that can be seen through a telescope, and a short history of the observation of these planets from Earth and using space probes. The bulk of the book is taken up by a series of small (about 10° by 14°) detailed maps of the nearside of the Moon. These are complimented by notes on the opposite page giving details of crater sizes and depths, and the occupation and country of origin of the dignitary the crater is named after. There is a ten-page section of Mars maps. The word colour in the title is rather misleading as all the maps are just brown with a blue border, they could equally well all have been black-and-white. Also, as the book is obviously geared to the observer with a small telescope, I think it would have been more useful to have the maps in 'telescope' coordinates with North at the bottom and East on the left. As it is, the book shows the Moon as it appears to the naked eye, with normal selenographic upright coordinates. These are, however, minor quibbles with what is clearly a well written, beautifully drawn, inexpensive book, a book which is bound to become a firm favourite with amateur astronomers.

I have before me four books by Patrick Moore, the genial presenter of the British Broadcasting Corporation's television series *Sky at Night*, which has continued monthly without a break since April 1957. As a populariser of astronomy and space science, Patrick Moore is without equal. His prolific pen produces books for the beginner in astronomy, books which read easily; in fact, books which read exactly as Moore speaks, with staccato, bubbling, infectious enthusiasm. *The Story of Astronomy* (Macdonald and Jane's: London; £5.95) was first published in 1961 and is now in its fifth, revised edition, having been brought completely up-to-date in both text and illustrations. It is an achievement for any

book to get to five editions; and reading this one, one can easily understand why it did. It is an ideal introduction to astronomy for the young reader; in fact, one could go so far as to say that every bright youngster should have a copy. It is clearly, and in many cases beautifully, illustrated. Moore starts with stone-age observations and tells the story of the stars, planets and galaxies right through to the time of the Viking landing on Mars.

Guide to the Comets (Lutterworth: Guildford, UK; £4.25) is a revised edition of Moore's 1973 book. It contains descriptions of the basic observed structure of comets, how they fit into the Solar System family, and how they are discovered by the enthusiastic band of comet hunters. Halley's comet and Encke's comet are discussed in detail, and the book ends with a discussion of lost comets and theories of cometary origins. Sixteen photographs are included, and the book has useful lists of known periodic comets. So far as it goes, the book is a good introduction but I can't help feeling that it would be greatly improved if it went slightly further. A 25% addition to the 96 pages could have touched on such topics as the way orbital parameters vary between cometary families, recent space-lab observations of cometary spectra, comet tail formation, comet decay and meteor stream formation, factors affecting the choice of comet nuclei models, and cometary outbursts.

Patrick turns his gaze on to the Red Planet in *Guide to Mars* (Lutterworth: Guildford, UK; £5.95). Again, the subject is approached historically, starting with the pre-1830 division of the Martian surface into dark areas and white polar caps, moving through the canal controversy of Schiaparelli and Lowell to the modern items of photography from Mariner Space probes and surface details, and the excitement of the search for life from the Viking craft. Mars has long been a world of conjecture; the canals have vanished into the realm of science fiction but the problem of life or no life is still very much with us. (Harping back to Heppenheimer, even Martian colonies are not beyond the bounds of possibility.) This book is a mine of information, and has many maps and photographs; I recommend it to anyone who wants a relatively painless introduction to the intricacies of this planet.

The Astronomy of Southern Africa (Robert Hale: London; £4.95) by Patrick Moore and Peter Collins, traces the history of South African astronomy from 1658 when Father Guy Tachard, a Jesuit priest en route for Siam, set up an observatory in Cape Town, to the present day when the country is liberally dotted with visual and radio observatories. Moore and Collins discuss the work of L'abbé de la Caille, who charted the positions of almost 10,000 southern stars

between 1751 and 1752, Sir John Herschel who set up a great telescope of 20-foot focal length in 1834, and Sir David Gill, one of the most outstanding of all the astronomers of South Africa, and many others. They also sketch the histories of the Royal Observatory Cape of Good Hope, the new Sutherland Observatory, the Government Meteorological Observatory and Union Observatory, Johannesburg, the Leiden Station, Hartebiespoort, the Yale Observatory and the Radcliffe, Pretoria. The illustrations (many from museums and archives), the many glimpses into the lives of the observers and the sensible discussions of their aims and achievements, make this book a joy.

While south of the equator, let us briefly discuss *Astronomy for the Southern Hemisphere* (Reed: Wellington, Sydney; Bailey: Folkestone, UK; £10.85) by Lionel Warner, which caters especially for star gazers in those parts. Mr Warner is obviously a very successful teacher, and his descriptions of some of the demonstrations that he uses in his classes come over very well. It is an excellent first book for the astronomical 'doer' as opposed to the astronomical reader. I found his monthly star maps, however, much too barren.

J. Hedley Robinson's *Astronomy Data Book* (Wiley: New York; £8.25; \$13.90) is rather like the curate's egg, only good in parts. I was very impressed by some of the tables listing, for example: brightest galaxies; variables observable with the naked eye over all and part of their brightness ranges; specific variable types, such as T Tauri, T Orionis, R Coronae and U Geminorum; binary stars; and the 286 brightest stars, all of which will be most useful to the amateur observer. Other tables such as, for example, a simple alphabetical list of formations on the far side of the Moon were much less useful. The glossary, with its very sharp, few word entries, I found to be distinctly unhelpful and in certain cases misleading.

Finally, we come to *The New Astronomy and Space Science Reader* (Freeman: San Francisco and Reading; hardback £12/\$15; paperback £5.50/\$7.50) edited by John C. Brandt and Stephen P. Maran. This book contains a collection of some 44 articles on current topics in optical, radio and space astronomy research both observational and theoretical, many of which are only briefly touched on in formal textbooks. These articles have been gleaned from such journals as *Natural History*, *Smithsonian*, *Scientific American*, *Science*, *Nature* and the *Astrophysical Journal*. The book provides a superb bridge between textbooks and the immense and rather frightening world of the professional journal. All amateur and student astronomers will gain immensely by reading it.

David W. Hughes

David W. Hughes is Lecturer in Physics and Astronomy at the University of Sheffield, UK.

obituary

Jacques Trefouel

PROFESSOR Jacques Trefouel, Director-General of the Pasteur Institute in Paris from 1940–1965 and a figure of great influence on the French scientific scene during this period and for many years after his retirement, died on 11 July 1977 at the age of 80.

He was an outstandingly capable and skilful organic-synthetic chemist who dedicated his whole active scientific life to the field of chemotherapy. He entered the Pasteur Institute at the age of 23 when he became a student of the great master of French chemotherapy, Ernest Fourneau, head of the Department of Chemotherapy created by the then Director-General of the Pasteur Institute, Emile Roux. A year later he married a colleague of his, Thérèse Boyer, who was not only an equally brilliant synthetic organic chemist, but also an exceptionally attractive lady of great charm and elegance. Nearly all his original papers were published jointly with his wife, and Jacques and Thérèse Trefouel became one of the most productive and best known scientific couples. They stayed at the Pasteur Institute until their statutory retirement.

Their first papers, most of them published jointly with E. Fourneau, followed Paul Ehrlich's lines and led to a series of substances active against trypanosomes, spirochetes and plasmodia.

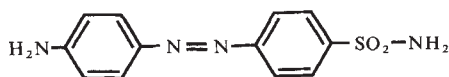
The most important contribution of the Trefouels made in collaboration with their brilliant colleagues the pharmacologist Daniel Bovet and the microbiologist Frederico Nitti, which earned them a position of immortality in the biomedical sciences, was the discovery in 1939 of the chemotherapeutic properties of sulphanilamide against bacterial infections.

During his extended histological studies, at the end of the last and the beginning of this century, Paul Ehrlich had become aware of the strong antibacterial properties of some of the synthetic aniline dyes, but unfortunately all of these were far too toxic for systemic chemotherapeutic use. However, Paul Ehrlich hoped, in view of the fact that dyes were capable of specifically staining some part of cells while leaving others unstained, and in view of his firm belief that the possibilities of synthetic organic chemistry were practically unlimited, that one day a synthetic dye would be discovered



which would specifically kill bacteria without affecting the host organism they had infected. He was encouraged in these thoughts by his finding that the dyes trypan blue and trypan red, were moderately effective in the chemotherapy of trypanosomal infections.

Stimulated by Paul Ehrlich's ideas the pharmaceutical divisions of the German dyestuff industries, then leading the world, made a tremendous effort to test thousands of dyes for chemotherapeutic efficiency in bacterial infection, but without success. In 1935 G. Domagk, of Bayer's, reported the sensational news that finally he had found an azodye, prontosil



which was capable of protecting mice against streptococcal infections and was also effective in man. This dye had been tested for over two years before its chemotherapeutic properties were released for publication. It thus seemed that Paul Ehrlich's ideas, after a period of over 30 years, were finally vindicated. However, the Trefouels, Bovet and Nitti proved conclusively a few months later in the same year that the chemotherapeutic properties in bacterial infections of prontosil had nothing to do with its dye nature, but resided totally in the non-coloured moiety sulphanilamide of the prontosil molecule. They postulated that prontosil was reduced to sulphanilamide in the animal body, an hypothesis which was later proved correct experimentally and explained why prontosil was inactive *in vitro*, but active *in vivo*.

There can be no doubt that the discovery of the chemotherapeutic

properties of sulphanilamide marked a major revolution in therapeutic medicine, both with regard to its practical use and theoretically. Sulphanilamide, synthesised as early as 1908, is a very simple molecule and can be readily modified. The Trefouels synthesised over a hundred derivatives, among them the sulphones which later were recognised to be valuable chemotherapeutic agents in the treatment of leprosy. Since then many thousands of sulphonamides have been synthesised in the laboratories of numerous pharmaceutical firms, and the advent of the antibiotics has not eclipsed them. They are still widely used in the clinic, particularly for the treatment of urinary infections caused by *E. coli* in combination with folic acid antagonists of 2,4-diamino pyrimidine structure (sulphanilamide itself was shown in 1940 by D. D. Woods in this country to antagonise the structurally similar para-amino benzoic acid, a part of the folic acid molecule, a discovery which, on its own, signified a major advance in the science of biochemistry).

Professor Trefouels scientific achievements were rapidly recognised and rewarded inside and outside the Pasteur Institute. He was promoted to Head of the Laboratory and, a few years later, to be Head of the Division of Chemotherapy. In 1940 he reached the administrative pinnacle of the Pasteur Institute when he was nominated Director-General. He was put into this prominent position while France was passing through some of the most difficult years of its history.

In 1939 World War II broke out and a year later France was conquered and humiliated by Germany. Jacques Trefouel, thanks to his inexhaustible spirit of enterprise, his great diplomatic talent and flexibility, his courage and his firm, deeply felt and uncompromising patriotism, managed to steer the Pasteur Institute successfully through very stormy waters with dignity and determination, and to preserve the essence of its unique historic scientific traditions and patrimony. He conceded nothing of importance of the possessions of the Pasteur Institute to the enemy, and his authority inside the Institute remained untarnished and undisputed.

He was a very courteous and polished personality, unruffled in situations of difficulty, and his radiant smile was irresistible. He was very erudite and wise, and was always very

kind and friendly, though he could be quite firm in relation to the people with whom he had to deal. He was gifted with a quick intelligence which enabled him to understand complex situations in a few moments, and he was endowed with a very refined, inimitable and typically French sense of humour.

His exceptional human qualities enabled him to keep on good terms with the majority of his senior colleagues and they made him popular among the staff of the Pasteur Institute. He appointed judiciously and, on the whole, successfully, new members of the staff and succeeded in maintaining the very high scientific standards of the Institute. He also created and maintained close contacts with the French pharmaceutical industry, to both its own benefit and that of the Pasteur Institute.

In my view, the Pasteur Institute never had a more dedicated and successful director than Jacques Tréfouël.

Throughout his onerous social duties as Director-General of the Pasteur Institute, Jacques Tréfouël received extensive, continuous and competent support from his wife who combined the properties of an excellent scientist with those of a perfect hostess. Time passed quickly during the animated and witty conversations covering a wide range of subjects at their elegant parties, which remained an unforgettable experience in the memory of those privileged to participate in them.

Jacques Tréfouël received numerous high honours from the French and other Governments and scientific societies. Among these were his nomination to the grade of a Grand Officer of the Legion d'Honneur, to the Membership and later Presidency of the French Academy of Sciences and the National Academy of Medical Sciences, many honorary degrees in the Universities of Europe, including Oxford and Cambridge in this country, and the Americas, and membership of many foreign academies and learned societies.

The passing of Jacques Tréfouël marks the end of an era in European science.

Ernst Chain

G. K. Green

GEORGE KENNETH GREEN, or Ken Green as he was known in the world of particle accelerators, died on 15 August 1977 of a heart attack while visiting his son at Brownsville, Texas. He was 66 years of age and had lived through, and taken a leading part in, one of the most extraordinary technological de-

velopments of our time.

Ken Green was born in St David, Illinois, and retained all his life the looks and the dry humour of a Mid-Westerner. He went to Illinois University, obtained a Ph.D. degree in physics there in 1937, and stayed on a year afterwards as an Associate in Physics. His first experience with what became his life's work was at the Radiation Laboratory of the University of California where he did design work on cyclotrons and nuclear physics research. It was there, at what was then the fountainhead of accelerator developments, under the inspiration and leadership of Ernest Lawrence, that Ken Green must have fallen in love with accelerators. He also spent a short time at the Department of Terrestrial Magnetism of the Carnegie Institution of Washington D.C.

He spent the war years with the Army Signal Corps, joining in 1942 as a second lieutenant, and in 1946 he was Army Electronic Representative and Technical Head of the Signal Corps Group at Operation Crossroads at Bikini. Later, he became principal physicist at the Evans Signal Laboratory of the Signal Corps at Belmar, New Jersey. During this period he worked on sonar and was one of the inventors of the proximity fuse, an important advance in firing rockets and artillery. At the Bikini test of atomic bombs he was involved in the development of instrumentation to study nuclear explosions. For his services during the war he received both the Civilian Distinguished Service Award and the US Army Legion of Merit.

Ken Green joined the Brookhaven National Laboratory in 1947 to work on the 'Cosmotron' and he never left that laboratory nor accelerator building for the rest of his life. The Cosmotron, in its time, was the largest accelerator in the world, and with a top energy of 3 GeV it was the first accelerator to exceed one thousand million electron volt particle energy. Ken Green was involved in the design and construction of every part of this machine. Formally, he was a senior scientist; in practice he combined a considerable design ability across the whole field of accelerator technology with a remarkable talent for supervising the construction of this giant machine—a talent rather rare in those days. The result was that Ken Green knew every detail of the Cosmotron, the reasoning behind its design, how it was constructed, and how well or badly it operated. No wonder that he earned the name of 'Mr. Cosmotron.'

The construction of the Cosmotron was finished in 1952, and many im-

provements were made to it subsequently, in all of which Ken Green was intimately involved. Meanwhile, another, even bigger accelerator project was being quietly conceived at Brookhaven, and in Europe a new international laboratory was being set up, called CERN, which aimed at building a similar machine. Then followed one of those unofficial and extremely effective collaborations between two laboratories which prove so beneficial to the course of scientific research, and Ken Green entered whole-heartedly into this collaboration.

The two machines, the 30 GeV AGS at Brookhaven and the 25 GeV CPS at CERN were essentially designed together. The principle of alternating gradient focusing was discovered at Brookhaven at this time, and the two teams worked out the consequences of this important idea together, and based the design of their machines on this new principle. Brookhaven staff came to Europe, CERN staff went to Brookhaven, and the success of these two machines owed much to the close collaboration between Ken Green's team at Brookhaven and the CERN team at Geneva.

The two machines came into operation at about the same time in 1960, and the two teams celebrated each other's success. The AGS and CPS were for many years the highest energy accelerators in the world. Ken Green became the Chairman of the Accelerator Department of the Brookhaven National Laboratory in 1960, having first served for a while as deputy-chairman under Leyland Haworth, and he held that post until 1970.

His last most notable contribution to accelerator design was the electron machine for the National Synchrotron Light Source which he started before his official retirement from Brookhaven Laboratory and continued for the short while afterwards until his death. Construction of this machine is due to start at Brookhaven towards the end of 1977. His last appearance in Europe was in the spring of this year when he came over to CERN to participate in the inauguration of the European 400 GeV SPS machine.

Ken Green has earned an immortal place in the ranks of the great accelerator builders with the Cosmotron and the AGS which were not only the highest energy machines of their time but also very challenging projects, involving many technological advances. All his many friends and colleagues throughout the world, in all the laboratories where these giant machines exist, will mourn his death, and remember with gratitude the help and encouragement he has given them.

J. B. Adams